TWENTY-FIFTH ANNUAL PROGRESS REPORT

For the Period
September 1, 2014 to August 31, 2015

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INTRODUCTION

The U.S. aquaculture industry generated nearly $1.4 billion for over 3,000 producers in 2013. Though minor in a global context, accounting for 0.6% of total world value, the domestic impact of U.S. aquaculture is substantial, accounting for approximately 181,000 jobs and generating an estimated $5.6 billion annually. Yet, anticipated growth in the industry, both in magnitude and in species diversity, continues to fall short of expectations.

Much of what is known about aquaculture science is a result of institutional attention given to our traditional capture of wild fisheries with the goal of releasing cultured fishes into public waters for enhancement of declining public stocks. Despite extensive efforts to manage wild populations for a sustained yield, as a nation we consume substantially greater amounts than we produce. Much of the United States' demand for seafood has been met by imports. The U.S. imports a majority of its fish and shellfish and, after Japan, is the world's second largest importer of seafood (valued at $17.6 billion in 2012). Fisheries imports are the largest contributor to the U.S. trade deficit among agricultural products.

Landings for most U.S. commercial capture fisheries species and recreational fisheries have been relatively stable during the last decade, with many fish stocks being overexploited. In this situation, aquaculture provides an opportunity to reduce the trade deficit and meet the rising U.S. demand for fish products. This can be achieved by a partnership of the Federal government, State and local public institutions, and the private sector with expertise in aquaculture development.

The U.S. Congress has stressed the importance of a strong domestic aquaculture industry to: (1) increase American production of fish and shellfish, (2) reduce dependence on foreign suppliers, and (3) benefit rural America by the development of alternative agricultural crops and creation of new jobs. Recognizing that the aquaculture industry cannot achieve full potential without strong national leadership and direction, the U.S. Congress created an opportunity for making significant progress in aquaculture development in 1980 by passage of the National Aquaculture Act -362). This act addressed the importance of a strong domestic aquaculture industry and established the Joint Subcommittee on Aquaculture (JSA). The JSA is an interagency body that is chaired by the Secretary of Agriculture. It has numerous responsibilities and is to provide coordination and recommendations for Federal aquaculture policy. The Congress also amended the National Agricultural Research, Extension, and Teaching Policy Act of 1977 in Title XIV of the Agriculture and Food Act of 1980 (P.L. 97-98) by granting authority to USDA to establish aquaculture research, development, and demonstration centers in the United States in association with colleges and universities, State Departments of Agriculture, Federal facilities, and non-profit private research institutions. Five such centers have been established: one in each of the northeastern, north central, southern, and western regions, and one in Hawaii. As used here, a Center refers to an administrative center. Centers do not provide monies for brick-and-mortar development.

Centers encourage cooperative and collaborative aquaculture research and extension educational programs that have regional or national application. Center programs complement and strengthen other existing research and extension educational programs provided by USDA and other public institutions. As a matter of policy, centers implement their programs by using institutional mechanisms and linkages that are in place in the public and private sector.

The mission of the RACs is to support aquaculture research, development, demonstration, and extension education to enhance viable and profitable U.S. aquaculture which will benefit consumers, producers, service industries, and the American economy. The North Central Regional Aquaculture Center (NCRAC) serves as a focal point to assess needs, establish priorities, and implement research and extension educational programs in the twelve state agricultural heartland of the United States. NCRAC also provides for coordination of interregional and national programs through USDA's National Coordinating Council for Aquaculture (NCCA). The council is composed of the RAC directors and USDA personnel.

ORGANIZATIONAL STRUCTURE

In the period of 1988 through 2011, Michigan State University (MSU) and Iowa State University (ISU) worked together to develop and administer programs of NCRAC through a memorandum of understanding. MSU was the prime contractor for the Center and had administrative responsibilities for its operation; ISU administered the extension/outreach activities for the Center. In 2012 NCRAC became solely administered by Iowa State University where the Office of the Director is now located. At the present time the staff of NCRAC at ISU includes Joseph E. Morris, Director; Denise Birney, Administrative Assistant; and D. Allen Pattillo, Program Extension Specialist.

The Center Director has the following responsibilities (0.65 FTE):

- Develop and submit proposals to USDA/NIFA which, upon approval, becomes a grant to the Center;
- Coordinate the development of research and extension projects including Work Group formation, review of project outlines for technical and scientific merit, feasibility, and applicability to priority problems and then submission to the Board of Directors for their approval after which, Board-approved project outlines are submitted to USDA/NIFA for approval in a Plan of Work or an Amendment to a Plan of Work;
- Oversee the development of appropriate agreements (sub- contracts) by the Administrative Assistant for purposes of transferring funds for implementation of all projects approved under the grants;
- Serve as executive secretary to the Board of Directors, responsible for preparing the agenda and minutes of Board meetings;
- Coordinate and facilitate interactions among the Administrative Center, Board of Directors, Industry Advisory Council (IAC), and Technical Committee/Research and Extension (TC/R and E);
- Monitor research and extension activities;
- Recruit other Administrative Center staff as authorized by the Board of Directors;
- Serve as an additional source of technical information for the regional aquaculture community;

- Maintain liaison with other RACs; and
- Serve on USDA's National Coordinating Council for Aquaculture.

The Center Director also has the following responsibilities (0.25 FTE) for extension/outreach responsibilities for the Center:

- Give regional presentations;
- Develop and distribute (including posting on the Web) news releases for new NCRAC publications;
- Supervise technical editors for NCRAC publications;
- Oversee the development of extension projects;
- Survey NCR aquaculture industry to guide future NCRAC extension programming; and
- Proofing of “final” draft of new NCRAC publications.

The Administrative Assistant (1.0 FTE) has the following responsibilities:

- Prepare correspondence;
- Maintain the administrative calendar, including scheduling of meetings and making travel arrangements;
- General office management;
- Answer or direct inquiries appropriately relating to aquaculture in general and the Center in particular;
- Maintain and monitor all budgetary matters for both the Center and sponsored projects including developing sub-contracts with other parties for purposes of transferring funds for implementing all approved projects;
- Compile information for periodic reports to the Center's Board of Directors and maintain records of Board business;
- Assist in preparation of Center reports to USDA/NIFA, including annual reports and plans of work;
- Maintain database of persons interested, involved with, or who should be kept informed of the Center's activities; and
- Monitor Web site and keep Director and Program Specialist updated on changes/additions.

The Program Extension Specialist (0.5 FTE) has the following responsibilities:

- Interaction with associated information technology staff NCRAC Web site and NCRAC List Serve (In cooperation with Regional Extension Specialist); Regional Extension Meetings;
- Coordination with other state extension contacts and the Regional Aquaculture Extension Specialist, Chris Weeks (Michigan State University), who cannot address all of the needs in all 12 states of the region equally well because of budgetary and time limitations;
- Regional presentations;
- Representation on NCRAC TC/E as Iowa’s representative on extension;
- Serve as Chair of NCRAC Extension Working Group committee;
- Preparation of impact statements resulting from NCRAC-funded extension projects;
- Maintain the NCRAC video collection and distribution;

- Initial editing of "final" draft of new NCRAC publications;
- Review and prepare responses to e- mail requests sent to NCRAC@iastate.edu;
- NCRAC mailings;
- Review of all current extension/outreach products for possible deletion or revision; and
- Help with technical and logistical support for the NCRAC Annual Program Planning Meetings.

The Board of Directors (BOD) is the primary policy-making body of the NCRAC. The BOD has established an Industry Advisory Council (IAC) and Technical Committee (TC). Membership of the BOD consists of four persons from the IAC, a representative each from the North Central Regional Association of State Agricultural Experiment Station Directors and the North Central Cooperative Extension Association, a member from a non-land grant university, representative from the university (Iowa State University) responsible for the Center, a member from a 1890 institution, and chairs of the two subcommittees of the Center's Technical Committee. The IAC is composed of representatives from each state's aquaculture association and six at-large members appointed by the BOD who represent various sectors of the aquaculture industry and the region as a whole. The TC is composed of a sub-committee for Extension (TC/E) and a sub-committee for Research (TC/R). Directors of the Cooperative Extension Service and Experiment Station Directors within the North Central Region appoint representatives to the TC/E and TC/R, respectively. The TC/R has broad regional make-up and is composed of scientists from universities and state agencies with varied aquacultural expertise who are appointed by the BOD. Each sub-committee of the TC has a chairperson who serves as a member of the BOD.

NCRAC functions in accordance with its *Operations Manual* which is periodically amended and updated with BOD approval. It is an evolving document that has changed as the Center's history lengthens. It is used for the development of the cooperative regional aquaculture and extension projects that NCRAC funds.

ADMINISTRATIVE OPERATIONS

Since the inception of NCRAC on February 1, 1988, the role of the Administrative Center has been to provide all necessary support services to the BOD, IAC, TC, and project work groups for the North Central Region as well as representing the region on the NCC. As the scope of the NCRAC programs expand, this has entailed a greater work load and continued need for effective communication among all components of the Center and the aquaculture community.

The Center functions in the following manner.

- After BOD approval of Administrative Center costs, the Center submits a grant to USDA/NIFA/Grants Management Branch for approval. To date the Center has received 25 grants from USDA for FY88 (Grant #88-38500-3885), FY89 (Grant #89-38500-4319), FY90 (Grant #90-38500-5008), FY91 (Grant #91- 38500-5900), FY92 (Grant #92-38500-6916), FY93 (Grant #93-38500-8392), FY94 (Grant #94-38500-0048), FY95 (Grant #95-38500-1410), FY96 (Grant #96-38500-2631), FY97 (#97-38500- 3957), FY98 (#98-

38500-5863), FY99 (#99-38500-7376), FY00 (#00-38500- 8984), FY2001 (#2001-38500-10369), FY2002 (#2002-38500-11752), FY2003 (#2003-38500-12995), FY2004 (#2004-38500-14269), FY2005 (#2005-38500- 15847), FY2006 (#2006-38500-16900), FY2007 (#2007-38500-18569), FY2008 (#2008-38500-19157), FY2009 (#2008- 38500-19157 extension) FY2010 (#2010-38500-20929), FY2011 (#2010- 38500-20929 Amendment), FY2012 (2012-38500-19550), FY2013 (#2012- 38500-19550 Amendment) and FY2014 (2014-38500-22138), FY2015 (#2014-38500-19550 Amendment) with monies totaling $20,286,818. Currently, four grants are active (FY12-15); the first 23 grants (FY88-10) have terminated, and the 2010 grant ended June 30, 2015.

- The Center annually coordinates a biannual program planning meeting which typically sets priorities for the next 2-year funding cycle and calls for development of project outlines to address priority problem areas.

- Work Groups are formed which submit project outlines to the Center. The projects are peer reviewed by experts from both within and outside the region and a Project Review Committee.
- The BOD, using the Project Review Committee's recommendation and reviewers' responses, decides which projects are to be approved and funding levels. The Center conveys BOD decisions to all Project Work Groups. Those that are approved for funding are asked to submit revised project outlines incorporating BOD, Project Review Committee, and reviewers' comments.
- The Center then submits the revised project outlines as a Plan of Work (POW) to USDA for approval.
- Once a POW is approved by USDA, the Center then prepares subcontracts for each participating institution. The Center receives all invoices for subcontractual agreements and prepares payment vouchers for reimbursement. Thus, Center staff serve as fiscal agents for both receiving and disbursing funds in accordance with all terms and provisions of the grants.

Through August 31, 2015, the Center has funded or is funding 107 projects through 515 subcontracts from the first 25 grants received. Funding for these Center- supported projects is summarized in Table 1 below (pages 8-11). Information about funded projects is also available at the Center's Web site (http://www.ncrac.org). During this reporting period, the Publications Office at ISU produced and distributed a number of publications including fact sheets, technical bulletins, and videos. A complete list of all publications from this office is included in the on-line Appendix under Extension.

Other areas of support by the Administrative Office during this reporting period included: monitoring research and extension activities and developing progress reports; developing liaisons with appropriate institutions, agencies and clientele groups; soliciting, in coordination with the other RACs, written testimony for the U.S. House Appropriations Subcommittee on Agriculture, Rural Development, Food and Drug Administration, and Related Agencies and the U.S. Senate Appropriations Subcommittee on Agriculture, Rural Development, and Related Agencies; participating in the NCA; numerous oral and written presentations to both professional and lay audiences; working with other fisheries and aquaculture programs throughout the North Central Region; and maintaining the NCRAC Web site.

PROJECT REPORTING

As indicated in Table 1, NCRAC has funded a number of projects for many of the project areas it has selected for research and extension activities. For example, there have been 22 separately funded projects in regard to Extension and 10 on Yellow Perch. Project outlines have been written for each separate project within an area, or the project area itself if only one project. These project outlines have been submitted in POWs or amendments to POWs for the grants as indicated in Table 1. Many times, the projects within a particular area are continuations of previously funded activities while at other times they are addressing new objectives. Presented below are Progress Reports for projects that were underway or completed during the period September 1, 2014 to August 31, 2015. Projects, or Project components, that terminated prior to September 1, 2013 have been reported on in earlier documents (e.g., 1989-1996 Compendium Report and other Annual Progress Reports). The following reports are placed in order of selected key word(s): Aquaculture Drugs, Baitfish, Conferences/Workshops, Crayfish, Economics/Marketing, Extension, Hybrid Striped Bass, Largemouth Bass, National Coordinator for Aquaculture, Nutrition/Diets, Other, Salmonids, Sunfish, Tilapia, Viral Hemorrhagic Septicemia, Walleye, Wastes/Effluents, and White Papers. In addition, the format style of these reports differs from previous years, e.g., inclusion of Project Summary and Impacts Summary.

A cumulative list of all publications, manuscripts, papers presented, or other outputs for all funded NCRAC project areas is located at http://ncrac.org

Table 1. North Central Regional Aquaculture Center-Funded Projects.

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Aquaculture Drugs	1	7/1/96-6/30/97	$27,000	95-38500-1410
	2	12/1/96-11/30/97	$950	95-38500-1410
	3	10/1/99-9/30/00	$8,415	97-38500-3957
	4	6/1/04-11/30/05	$223,677	2003-38500-12995
	5	7/15/04-7/14/05	$60,000	2003-38500-12995
	6	11/1/04-10/31/06	$50,000	2002-38500-11752
	7	1/1/06-12/31/06	$129,936	2005-38500-15847
	8	9/1/08-8/31/10	$150,000	2008-38500-19157
	9	9/1/09-8/31/10	$27,880	2008-38500-19157
	10	9/1/11-8/31/31	$100,000	2010-38500-20929
	11	9/1/12-8/31/14	$240,000	2012-38500-19550
			$1,017,858	
Baitfish	1	9/1/92-8/31/94	$61,973	92-38500-6916
	2	9/1/06-8/31/08	$111,997	2006-38500-16900
			$88,003	2005-38500-18547
			$261,973	
Conferences/Workshops/Symposia				
Environmental Strategies Symposium	1	9/1/00-5/31/01	$5,000	96-38500-2631
Nat'l. Aquaculture Ext. Workshop/Conference	1	10/1/91-9/30/92	$3,005	89-38500-4319
	2	12/1/96-11/30/97	$3,700	95-38500-1410
	3	11/1/02-10/31/03	$4,500	00-38500-8984
	4	1/1/06-12/31/06	$5,000	2005-38500-18547
	5	9/1/10-8/31/11	$5,000	2008-38500-19157
			$21,205	
NCR Aquaculture Conference	1	6/1/90-3/31/91	$7,000	90-38500-5008
	2	12/9/98-6/30/99	$3,000	96-38500-2631
			$10,000	
Percis III	1	11/1/02-10/31/03	$4,000	00-38500-8984
Crayfish	1	9/1/92-8/31/94	$49,677	92-38500-6916
Economics/Marketing	1	5/1/89-12/31/91	$127,338	88-38500-3885
			$34,350	89-38500-4319
	2	9/1/91-8/31/92	$53,300	91-38500-5900
	3	9/1/93-8/31/95	$40,000	93-38500-8392
	4	9/1/99-8/31/01	$47,916	97-38500-3957
	5	9/1/03-8/31/04	$50,000	2002-38500-11752
	6	9/1/10-8/31/11	$23,565	2010-38500-20929
	7	9/1/12-8/31/14	$18,810	2012-38500-19550
			$451,745	
Extension ("Base" Extension—Project Nos. 1-15; Aquaculture Regional Extension Facilitator [AREF]—Project No. 17; and	1	5/1/89-4/30/91	$39,221	88-38500-3885
			$37,089	89-38500-4319
	2	3/17/90-8/31/91	$31,300	89-38500-4319
	3	9/1/91-8/31/93	$94,109	91-38500-5900
	4	9/1/93-8/31/95	$110,129	91-38500-5900

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Regional Aquaculture Extension Specialist [RAES]—Project Nos. 18-22	5	9/1/95-8/31/97	$10,813	92-38500-6916
			$20,391	95-38500-1410
	6	9/1/97-8/31/99	$38,000	97-38500-3957
	7	9/1/99-8/31/01	$94,000	99-38500-7376
	8	9/1/01-8/31/03	$28,500	99-38500-7376
			$18,154	2001-38500-10369
	9	9/1/03-8/31/05	$28,000	2002-38500-11752
	10	9/1/05-8/31/07	$211,545	2003-38500-12995
			$7,735	2005-38500-15847
	11	9/1/07-8/31/09	$21,850	2006-38500-16900
			$92,469	2007-38500-18469
	12	9/1/08-8/31/10	$37,966	2007-38500-18469
			$22,539	2008-38500-19157
	13	9/1/09-8/31/11	$29,000	2008-38500-19157
	14	9/1/11-8/31/13	$35,700	2010-35800-20929
	15	9/1/13-8/31/15	$45,000	2012-38500-19550
	16	9-1-15-8-31-16	$23,175	2012-38500-19550
	17	9/1/03-8/31/05	$100,000	2002-38500-11752
	18	9/1/05-5/31/09	$199,624	2004-38500-14269
	19	9/1/09-8/31/11	$150,000	2008-38500-19157
	20	9/1/11-8/31/13	$196,612	2010-38500-20929
	21	9/1/13-8/31/14	$101,280	2012-38500-19550
	22	9/1/14-8/31/16	$103,347	2014-38500-22138
			$1,927,548	
Hybrid Striped Bass	1	5/1/89-8/31/91	$68,296	88-38500-3885
			$68,114	89-38500-4319
	2	6/1/90-8/31/92	$101,000	90-38500-5008
	3	9/1/91-8/31/93	$96,550	91-38500-5900
	4	9/1/93-8/31/95	$168,000	93-38500-8392
	5	9/1/95-8/31/97	$150,000	95-38500-1410
	6	6/1/99-5/31/00	$15,000	96-38500-2631
	7	9/1/01-5/31/04	$98,043	98-38500-5863
			$211,957	2001-38500-10369
			$976,960	
Largemouth Bass	1	9/1/05-8/31/07	$170,000	2004-38500-14269
	2	9/1/14-8/31/16	$155,000	2014-38500-22138
			$325,000	
National Coordinator for Aquaculture INADs/NADAs	1	9/1/93-8/31/94	$2,000	89-38500-4319
		5/15/95-5/14/96	$5,000	94-38500-0048
		5/15/96-5/14/97	$6,669	92-38500-6916
			$3,331	95-38500-1410
		5/15/97-5/14/98	$15,000	96-38500-2631
		5/15/98-5/14/99	$13,241	94-38500-0048
		5/15/99-5/14/00	$10,000	95-38500-1410
	2	7/15/04-7/14/05	$9,000	2003-38500-12995
		9/15/05-8/31/06	$15,000	2004-38500-14269
		9/1/06-8/31/08	$40,000	2006-38500-16900
		5/15/08-5/14/09	$25,000	2007-28500-18469
			$144,241	

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
Nutrition/Diets	1	9/1/04-8/31/06	$200,000	2002-38500-11752
	2	9/1/07-8/31/09	$80,000	2006-38500-16900
	3	9/1/09-8/31/11	$80,000	2008-38500-19157
	4	9/1/10-8/31/12	$124,400	2008-38500-19157
	5	9/1/12-8/31/13	$75,000	2010-28500-20929
			$559,400	
Other				
Feed Training Carnivorous Fish	1	9/1/06-8/31/08	$165,446	2005-38500-15847
			$134,554	2006-38500-16900
			$300,000	
Snail Management/Grub Control	1	9/1/07-8/31/09	$225,000	2007-38500-18469
RAS Microbial Communities	1	9/1/09-8/31/10	$65,000	2008-38500-19157
Assessment of Winter Kill in Ponds	1	9/1/11-8/31/13	$175,000	2008-38500-19157
			$240,000	
Salmonids	1	6/1/90-8/31/92	$9,000	89-38500-4319
			$120,799	90-38500-5008
	2	9/1/92-8/31/94	$149,997	92-38500-6916
	3	9/1/94-8/31/96	$199,290	94-38500-0048
	4	9/1/97-8/31/99	$158,656	97-38500-3957
			$637,742	
Sunfish	1	6/1/90-8/31/92	$130,758	90-38500-5008
	2	9/1/92-8/31/94	$149,799	92-38500-6916
	3	9/1/94-8/31/96	$173,562	94-38500-0048
	4	9/1/96-9/31/98	$199,921	96-38500-2631
	5	9/1/99-8/31/01	$199,748	99-38500-7376
	6	9/1/13-8/31/15	$160,000	2012-38500-19550
			$1,013,788	
Tilapia	1	9/1/96-8/31/98	$118,791	96-38500-2631
	2	9/1/98-8/31/00	$150,000	98-38500-5863
			$268,791	
Viral Hemorrhagic Septicemia (VHS)	1	9/1/08-8/31/10	$197,960	2008-38500-19157
Walleye	1	5/1/89-8/31/91	$177,517	89-38500-4319
	2	6/1/90-8/31/92	$111,657	90-38500-5008
	3	9/1/91-8/31/92	$109,223	91-38500-5900
	4	9/1/92-8/31/93	$75,000	89-38500-4319
	5	9/1/93-8/31/95	$150,000	93-38500-8392
	6	9/1/95-8/31/97	$117,395	94-38500-0048
			$59,835	95-38500-1410
	7	9/1/99-6/30/02	$127,000	98-38500-5863
			$927,627	
Wastes/Effluents	1	9/1/92-8/31/94	$153,300	92-38500-6916
	2	9/1/96-8/31/98	$100,000	96-38500-2631
	3	9/1/01-8/31/04	$106,186	00-38500-8984
			$88,814	2001-38500-10369
			$448,300	

Project Area	Project Number	Proposed Duration Period	Funding Level	Grant Number
White Papers	1	7/1/98-12/31/98	$4,999	96-38500-2631
	2	9/1/99-12/31/99	$17,495	97-38500-3957
			$22,494	
Yellow Perch	1	5/1/89-8/31/91	$76,957	88-38500-3885
			$85,7231	89-38500-4319
	2	6/1/90-8/31/92	$92,108	90-38500-5008
	3	9/1/91-8/31/93	$99,997	91-38500-5900
	4	9/1/93-8/31/95	$150,000	93-38500-8392
	5	9/1/95-8/31/97	$199,507	95-38500-1410
	6	9/1/97-8/31/99	$185,458	97-38500-3957
	7	9/1/98-8/31/00	$92,370	98-38500-5863
	8	9/1/01-5/31/04	$326,730	00-38500-8984
			$125,016	2001-38500-10369
	9	9/1/10-8/31/13	$150,000	2010-38500-20929
	10	9/1/13-8/31/15	$190,000	2012-38500-19550
			$1,773,866	
TOTAL			$11,810,175	

PROJECT REPORTS

Project Title: Drug Approval Research on 17 α-Methyltestosterone (Official Transfer of 17 α- Methyl testosterone [MT] Analytical Method for Feed) [Termination Report]
Key Word(s): Aquaculture Drugs
Dates of Work: September 1, 2009-August 31, 2015.
Total Funds Committed: $54,615
Participants: Mark P. Gaikowski, USGS, Upper Midwest Environmental Sciences Center, Wisconsin; Nilmini Wijewickreme, Reference Laboratory, CANTEST Ltd., British Colombia
Extension Liaison: Kevin Fitzsimmons, University of Arizona
Industry Liaison: Mark Willows, Binford Eagle Fisheries, North Dakota
Reason for Termination: Project objectives completed and funds have been terminated.

Project Objectives

1. Develop study protocols to conduct the MT feed method transfer of the MT analytical feed method.
2. Submit method transfer study protocols to the Center for Veterinary Medicine (CVM) for concurrence.
3. Provide final study protocols to participating laboratories.
4. Prepare and ship medicated feed to participating laboratories.
5. Assay control and medicated feed samples according to the study protocols concurred with by CVM.
6. Complete report of analysis and submit along with raw data to the Upper Midwest Environmental Sciences Center (UMESC).
7. Compare and discuss the results of both the CANTEST, Ltd. (CANTEST) reference (expert) and transferred (naïve) analyses of the MT transfer study samples based on the MT analytical feed method developed by the University of Wisconsin-Madison (UW- Madison).
8. Determine whether any changes are needed to the MT analytical feed method developed UWMadison based on the results of the MT feed transfer study.
9. Validate that the naïve analyst at CANTEST can analyze the MT feed samples according to the analytical feed method developed by UW-Madison.
10. Compile Final Study Report (FSR), archive raw data, and submit FSR to CVM through the UMESC MT investigational new animal drug (INAD) exemption.
11. Respond to CVM comments.
12. Gain acceptance from CVM for the MT feed method transfer study.

Project Summary

The approval of 17α-methyltestosterone (MT) medicated feed for use in tilapia to produce male fish would be of significant benefit to the industry. Tilapia is now the fifth most consumed seafood in the United States. Male fish grow faster than do their female counterparts, and by using all male fish, reproduction can be minimized or eliminated in grow-out systems, further benefiting growers. Approval of MT will allow all tilapia producers to have legal access to MT without an investigational new animal drug permit and will provide them with a legal means to yield increased biomass, thus resulting in more revenue for those producers. The production of male populations of tilapia is important to the U.S. tilapia industry if they are to remain competitive with foreign producers of tilapia. The remaining data requirements necessary for MT approval include a method transfer trial where

naive and experienced laboratories process MT medicated feed. Data from the sources must match for the transfer trial to be successful, and the method to be accepted by CVM.

Technical Summary and Analysis
Objective #1: Protocol was developed.

Objective #2: Protocol was accepted by CVM.

Objective #3: Protocol submitted to participating laboratory.

Objective #4: Prepare and ship medicated feed to participating laboratories. Feed was shipped.

Objective #5: Feed samples were analyzed by the participating laboratory.

Objective #6: Reports from expert and naive analysts were received from the participating laboratory.

Objective #7 All data were reviewed.

Objective #8: The method was revised and a new standard operating procedure developed.

Objective #9: Study was conducted between December 2009 and June 2010.

Objective #10: Final report was compiled and the report and all associated data submitted to the UMESC archives and to CVM for review.

Objective #11: Respond to CVM comments. UMESC made a final response to CVM in July 2015 addressing all CVM comments.

Objective #12: All data were accepted by CVM.

Principal Accomplishments
The production of male tilapia populations is critical to the U.S. tilapia industry if producers are to remain competitive with foreign tilapia producers. Approval of 17-α methyltestosterone (MT)-medicated feed for use in tilapia to produce greater than 80% phenotypic male populations would be of significant benefit to U.S. producers because male tilapia generate more biomass with less effort and time making them more cost efficient to raise.

A data requirement needed for the approval of an original new animal drug application (NADA) for MT use in tilapia is a requirement to validate the method for determining MT concentrations in fish feed containing MT. The validation requirement is termed a method transfer study where analysts naïve to the method procedures must fulfill method performance criteria when

analyzing fish feed samples with the method.

The study was conducted in two phases, a familiarization phase and a method transfer trial phase. During the familiarization phase, analysts in the participating laboratory naïve to the method procedures successfully performed the method (met method performance criteria) when analyzing control feed and control feed fortified with MT at nominal concentrations of 30, 60, and 90 µg/g. During the method transfer phase, analysts in the reference laboratory experienced with the method procedures and analysts of the participating laboratory successfully performed the method when analyzing control feed, control feed fortified with MT at nominal concentrations of 30, 60, and 90 µg/g, and feed containing MT at a nominal concentration of 60 µg/g (Table 1).

The method for determining MT concentrations in fish feed marketed with the brand name Masculinizing Feed for Tilapia® was validated meaning the method fulfilled the method robustness criteria of a method transfer trial. Table 1. Summary of select results and performance criteria from reference and participating laboratory analysts processing control feed, control feed fortified with 17-α methyltestosterone (MT) at nominal concentrations of 30, 60, and 90 µg/g, and MT medicated feed. Parameter Reference laboratory results include Acceptance criteria Calibration curve linearity (R2) 0.99995 and 0.99991 0.99998 and 0.99999 ≥ 0.9950 MT concentration in control feed < 15 µg/g < 15 µg/g < 15 µg/g. Accuracy (mean % recovery) of MT from fortified feed 84.9% – 86.9% 96.9% – 105% 80 – 110% Precision (%RSD) from 2 days where five samples of MT medicated feed were analyzed each day 2.3% and 1.9% 1.7% and 8.4% < 10%.

Impacts

The industry now has a CVM accepted method to determine 17-MT concentrations in fish feed. The method will be used to verify 17-MT concentrations in fish feed when the medicated feed is produced for the fish farmer.

Recommended Follow-Up Activities

No follow up studies are projected

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Aquaculture Drugs activities.

Technical Update

Table 1. Summary of select results and performance criteria from reference and participating laboratory analysts processing control feed, control feed fortified with 17-α methyltestosterone (MT) at nominal concentrations of 30, 60, and 90 µg/g, and MT medicated feed.

Parameter	Reference laboratory results	Participating laboratory results	Acceptance criteria
Calibration curve linearity (R^2)	0.99995 and 0.99991	0.99998 and 0.99999	$\geq$ 0.9950
MT concentration in control feed	< 15 µg/g	< 15 µg/g	< 15 µg/g
Accuracy (mean % recovery) of MT from fortified feed	84.9% – 86.9%	96.9% – 105%	80 – 110%
Precision (%RSD) from 2 days where five samples of MT medicated feed were analyzed each day	2.3% and 1.9%	1.7% and 8.4%	< 10%

Project Title: Effectiveness Research Leading to Approvals for Controlling Mortality in Coolwater and Warmwater Finfish due to Aeromonad Infections with Terramycin 200 for Fish® (oxytetracycline dehydrate) and Aquaflor® (florfenicol) [Progress Report]
Key Word(s): Aquaculture Drugs
Total Funds Committed: $150,000
Initial Project Schedule: September 1, 2008 to July 31, 2012
Current Project Year: September 1, 2014 to August 31, 2015
Participants: Mark P. Gaikowski, USGS, Upper Midwest Environmental Sciences Center, Wisconsin
Extension Liaison: Joseph E. Morris, Iowa State University
Industry Liaison: Mark Willows, Binford Eagle Fisheries, North Dakota

Project Objectives

1. Identify the etiologic agent (*Aeromonas* spp.) from isolates collected from disease outbreaks in the NCR and characterize the disease syndrome before conducting any effectiveness studies.
2. Have active, established Investigational New Animal Drug (INAD) exemptions or work with the sponsors of publicly disclosable INADs for Terramycin 200 for Fish® and Aquaflor®.
3. Develop draft pivotal effectiveness study protocols with the concurrence of the two drug sponsors (Phibro Animal Health=PAH for Terramycin 200 for Fish® and Schering- Plough Animal Health=SPAH for Aquaflor®).
4. Submit the draft pivotal effectiveness study protocols through established INADs for Terramycin 200 for Fish® and Aquaflor® for protocol concurrence from the CVM before beginning the effectiveness studies.
5. Conduct pivotal effectiveness studies on Terramycin 200 for Fish® and Aquaflor® according to Good Clinical Practice and the CVM concurred protocols.
6. Analyze the effectiveness data and prepare draft final study reports for Terramycin 200 for Fish® and Aquaflor® no more than four months after the studies are completed.
7. Submit the respective draft study reports to PAH and SPAH for their review.
8. Submit the final study reports through established INADs for Terramycin 200 for Fish® and Aquaflor® to CVM for acceptance no more than two months after PAH and SPAH have completed their reviews of the draft study reports.
9. Ensure that all questions and concerns about the final study reports are answered no more than one month after receiving comments from CVM.
10. If CVM accepts the data as proving effectiveness for the aeromonad infections encountered in the NCR, provide the acceptance letter and effectiveness studies to PAH and SPAH so that they can pursue supplemental NADA approvals for their respective drug products.

Project Summary
The efficacy of Terramycin 200 for Fish®- or Aquaflor®-medicated feed therapy to control mortality associated with motile aeromonad infections was evaluated in muskellunge and walleye under field conditions at Spirit Lake Fish Hatchery, a state walleye, northern pike, and muskellunge hatching and rearing station production facility in Spirit Lake, Iowa. The hatchery historically experiences rising mortality rates due to motile aeromonad septicemia as the water temperature rises in early July. Parameters evaluated included daily mortality, clinical observations, feed consumption, and water chemistry observations.

Anticipated Benefits
The data from this study, if accepted by CVM, will support the potential approval of oxytetracycline dehydrate (OTC) and florfenicol (FFC) medicated feed for use in cool- and warm-water fish to prevent mortality due to motile *Aeromonas septicemia*.

Project Progress
Objectives 1-5. — Complete

Objective 6. — Final report for musky trial is undergoing peer- review. Final report for walleye trial is in preparation.

Objectives 7-10. — No Progress.

Target Audiences
Aquaculturists rearing cool and warm water fish species who have the need to control mortality associated with mesophilic or motile *Aeromonas* infections (MAI).

Outreach Overview
UMESC will complete final reports when the *Aeromonas* species in the remaining isolates are identified and confirmed. Final reports will be disseminated to CVM, Phibro Animal Health and Merck Animal Health.

Deliverables (Outputs)
None.

Outcomes/Impacts
The control of mesophilic or motile *Aeromonas* infections is extremely relevant to the aquaculture industry as it has experienced income losses in food, sport and bait fish facilities due to MAI. The results from this project will directly affect the potential for approval of oxytetracycline dihydrate and florfenicol by the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM). The data from this study, if accepted by CVM, will support the potential approval of OTC and FFC medicated feed for use in cool- and warm-water fish to prevent mortality due to motile *Aeromonas septicemia*.

Impacts Summary

Relevance — The control of mesophilic or motile *Aeromonas* infections is extremely relevant to the aquaculture industry as it has experienced income losses in food, sport and bait fish facilities due to MAS.

Response — Pivotal studies were completed to assess the effectiveness of OTC and FFC in reducing mortality due to MAS in juvenile muskellunge and walleye.

Results— Results are being compiled.

Recap — Results are pending.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Aquaculture Drugs activities.

Technical Update

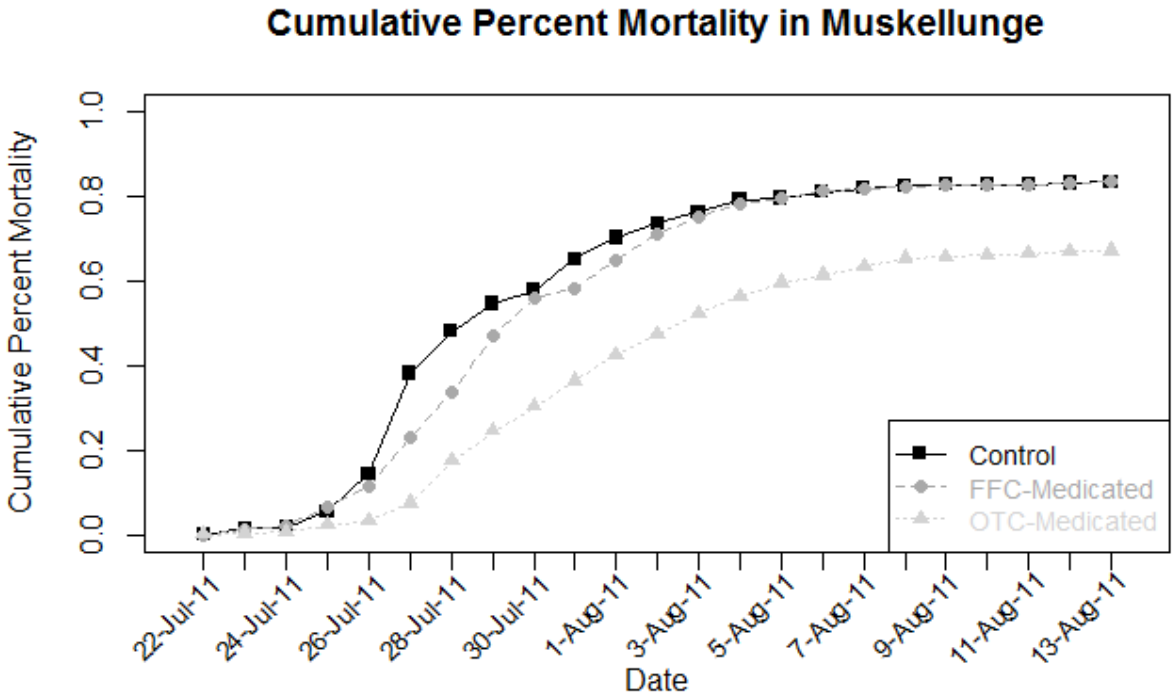

Figure 1. Cumulative percent mortality in muskellunge. Cumulative percent mortality of muskellunge offered non-medicated control feed, FFC-medicated feed at a nominal dose of 15 mg FFC/kg bodyweight/d, or OTC-medicated feed at a nominal dose of 82.5 mg OTC/kg BW/d for 10 days. Mortality was associated with motile aeromonad infection.

Project Title: Efficacy of Eugenol (AQUI-S®20E) to Reduce Transport Stress and Mortality of Tilapia and Yellow Perch [Progress Report]
Key Word(s): Aquaculture Drugs
Total Funds Committed: $100,000
Initial Project Schedule: September 1, 2011 to August 31, 2013
Current Project Year: September 1, 2014 to August 31, 2015
Participants: Mark P. Gaikowski, USGS Upper Midwest Environmental Sciences Center, Wisconsin; Christopher F. Hartleb, University of Wisconsin – Stevens Point, Wisconsin
Industry Liaison: Mark Willows, Binford Eagle Fisheries, North Dakota

Project Objectives

1. Interact with CVM to determine the study design and protocol needed to develop the effectiveness data to support a transport sedative claim for eugenol for selected finfish species. The protocol must comply with current CVM Guidance For Industry for the development of pivotal effectiveness data and the study data collection must with CVM Good Clinical Practices regulations.
2. Obtain fully disclosable Investigational New Animal Drug (INAD) exemptions for the selected sedative to be tested from CVM.
3. Obtain Categorical Exclusions from the requirement to complete an Environmental Assessment or complete an Environmental Assessment for the selected sedative prior to its use and receive concurrence from CVM Environmental Safety Team.
4. Submit the pivotal effectiveness protocol to CVM for concurrence.
5. Conduct pivotal effectiveness studies using the selected sedative on finfish species according to the CVM-concurred protocol and in compliance with CVM Good Clinical Practices regulations.
6. Summarize the study data into a Final Study Report (FSR) and archive all study data in publicly accessible archives
7. Submit the FSR to the publicly disclosable INAD file provided by CVM and request CVM review of the FSR and concur that the effectiveness technical section is complete for the selected sedative.
8. Respond to CVM comments on the FSR to ultimately obtain concurrence that the effectiveness technical section is complete for the use of the selected sedative as a transport sedative for the selected species.
9. Prepare a Freedom of Information summary of the submitted data and provide it to CVM.

Project Summary

Fish transport costs are a substantial portion of the operational expenses in the aquaculture industry in the North Central Region (NCR), especially as fuel costs continue to increase. Increasing fish loading density during transport could substantially increase the efficiency of NCR aquaculture operations by enabling the transport of more fish per gallon of fuel.

Anticipated Benefits

Transporting more fish per gallon of fuel would directly reduce operational costs resulting from less fuel being purchased and consumed. Additionally, fewer staff days would be required for transport and hauling with increased loading density. Reducing transport-mediated stress in fish could also improve market sales, especially at live market (either for food fish or baitfish) by

improving fish quality and appearance by reducing physical damage of fish during transport and decreasing post-transport disease occurrence. Reducing transport-mediated fish stress may also enhance fillet quality in fish transported to slaughter markets by reducing aerobic metabolism during transport, potentially improving fillet quality by maximizing residual energy stores in the fillet. When hauling juvenile fish for stocking, potential benefits would be realized by increasing loading density during transportation and maintaining acceptable post- transport survival.

Project Progress

Objective 1. — Upper Midwest Environmental Science Center (UMESC) collaborated with Center for Veterinary Medicine (CVM) and developed an acceptable protocol and study design for generating non-pivotal effectiveness data. UMESC submitted the protocol to CVM through the UMESC publicly-disclosable Investigational New Animal Drug (INAD) permits for AQUI-S®20E and requested an informal CVM review prior to conducting the study. CVM staff were uncertain about how to assess a potential label claim and data generated through non-pivotal effectiveness trials would be important for development of a pivotal effectiveness study. Data and reports from non-pivotal effectiveness trials and a draft pivotal effectiveness protocol were submitted to CVM in January of 2014. Responses from CVM regarding these submissions were received May 2014. Briefly, CVM response indicated that non-pivotal data informed them on both effectiveness and target animal safety. Suggestions concerning future studies under these conditions were to use fewer response variables. Itemized comments for revisions of the pivotal effectiveness protocol were addressed to work toward protocol concurrence from CVM and a revised pivotal effectiveness protocol was submitted in February 4, 2015. On March 27, 2015, CVM did not concur with the revised protocol submission. Although CVM largely did not have any qualms with overall study design, they could not concur with the protocol because no official label claim was put forth by AQUI-S New Zealand specifying the use of AQUI-S20E® for fish transport. No further protocol concurrence will be issued by CVM until a label claim is developed; regardless of their agreement with study design.

Objective 2. — All protocols, data, and final study reports submitted to CVM will be submitted by UMESC to INAD 011-766.

Objective 3. — Work within this objective is dependent on progress made by the drug sponsor on completion of an original Environmental Assessment for the use of AQUI-S® 20E.

Objective 4. — A revised pivotal effectiveness protocol was submitted based on input from CVM, UMESC and the drug sponsor (AQUI-S New Zealand Ltd.). The CVM non-concurrence letters were received. See description in Objective 1.

Objective 5. — Pivot effectiveness study was completed in Summer 2015. However, protocol concurrence with study design was not attained prior to study initiation for reasons described in Objective 1.

Objective 6. — A final study report is being prepared and its associated data audited by the UMESC QA Officer before review and acceptance by UMESC management. The drug sponsor has a maximum of 60 days to provide review comments to UMESC before the complete final study report and all trial data are archived and the FSR will then submitted to CVM through the UMESC (INAD 011-766).

Objective 7. — UMESC will submit the FSRs and associated data to CVM. Included with the submission will be appropriate correspondence and CVM- mandated forms to request CVM review to determine whether the submitted data support the potential approval of eugenol as a sedative to improve fish transport loading density without increasing post-transport mortality.

Objective 8. — UMESC will address specific study related issues identified by CVM in the review letter with an amended final report if needed.

Target Audiences

There are two targeted audiences: 1) directly, the manufacturer of AQUI-S®20E will gain knowledge in species- specific effects and applied concentration parameters for the commercial product of 10% eugenol, also, the CVM will use this knowledge as they draft action regarding the use of AQUI- S®20E as an immediate release finfish sedative; and 2) indirectly, fish haulers/transporters and aquaculturists will benefit should AQUI S®20E be approved for use when hauling yellow perch and tilapia as it could potentially support greater loading densities during transport.

Outreach Overview

Multiple presentations about the methods and results from the effectiveness studies were given to state, regional, and national aquaculture groups. This included presentations to the Wisconsin Aquaculture Association, Mid-Continent Warm Water Fish Culture Workshop, the North Central Regional Aquaculture Center, and the USFWS Aquatic Animal Drug Approval Partnership. Also, the final report for this project will be submitted to NCRAC for public distribution and parts of the effectiveness studies will be published in peer-reviewed scientific journals for broad public distribution (one manuscript currently accepted by journal).

Deliverables (Outputs)

None to date.

Outcomes/Impacts

Results of the effectiveness studies resulted in changes in knowledge based on the following:

1. Concentrations of AQUI-S®20E ranging from 200-300 mg/L resulted in multiple levels of sedation (including light sedation) and >95% mean survival 7 days post-transport for yellow perch at loading densities up to 360 g/L (three times the industry standard) in 17^{o}C (63 ^{o}F) water. Tilapia held at 22^{o}C (72 ^{o}F) showed signs of sedation for less than 4 h when exposed to AQUI- S®20E concentrations up to 300 mg/L, but had high mean survival (>90%) following a 10 h static exposure at a loading density of 480 g/L (two

times the industry standard).

2. Concentrations of AQUI-S®20E ranging from 200-300 mg/L were effective at reducing metabolic rates for yellow perch in 17^{O}C (63 ^{O}F) water relative to unsedated control fish.

Tilapia exposed to 300 mg/L AQUI-S®20E at 22^{O}C (72 ^{O}F) had significantly reduced metabolic rates relative to control fish at a loading density of 120 g/L (1 lb/gal). Results indicated that AQUI-S®20E sedation may benefit yellow perch at high loading densities during transport due to a reduction in metabolic rates, while further research is needed to assess the benefits of AQUI-S®20E sedation for tilapia at densities greater than 120 g/L (1 lb/gal).

Impacts Summary

Relevance.— Fish transport costs are a substantial portion of the operational expenses of the aquaculture industry in the North Central Region (NCR), especially as fuel costs continue to increase. Fish haulers/transporters are interested in any means that increase fish loading densities in hauling tanks to make deliveries more efficient.

Response. — Increasing fish loading density during transport could substantially increase the efficiency of NCR aquaculture operations by enabling the transport of more fish per gallon of fuel. Effectiveness studies were conducted to examine the sedation effect of AQUI-S®20E on yellow perch and tilapia as a means of decreasing fish respiration while increasing fish loading densities and thereby maximizing hauling efficiencies.

*Results***. —** Knowledge gained from the effectiveness studies included species- specific fish loading densities for yellow perch based on sedation effects and respirometry data. Results from the tilapia studies showed minimal changes in metabolic rates and sedation under simulated transport conditions and suggested that further studies are needed to characterize the response of tilapia to AQUI- S®20E during transport at high loading densities. These results are directly applicable to the manufacturer of AQUI- S®20E and the CVM as they evaluate AQUI-S®20E as an immediate release finfish sedative.

Recap. — AQUI-S®20E has the potential to allow fish haulers to transport fish at increased loading densities though the sedation effects were more pronounced for yellow perch than tilapia.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-funded Animal Drugs activities.

Project Title: Probiotics in Yellow Perch and Tilapia Culture [Progress Report]
Key Word(s): Aquaculture Drugs
Total Funds Committed: $240,000
Initial Project Schedule: September 1, 2012 to August 31, 2014
Current Project Year: September 1, 2014 to August 31, 2015
Participants: Konrad Dabrowski, The Ohio State University, Ohio; Timothy Johnson, University of Minnesota, Minnesota; Nicholas Phelps, University of Minnesota, Minnesota; Zhongtang Yu, The Ohio State University, Ohio
Extension Liaison: Nicholas Phelps, University of Minnesota, Minnesota
Industry Liaison: William Lynch, Millcreek Aquaculture. Marysville, Ohio

Project Objectives:

1. Characterize the microbial community of early ontogeny of yellow perch and tilapia during growout phase in control (laboratory) setting and compare to practical industry conditions (minimum of 2 farms for each species).
2. Isolate bacteria that possess the characteristics resulting in inhibition of pathogenic *Vibrio* and *Aeromonas* species.
3. Compare commercial probiotics to those isolates identified in Objective 2.
4. Establish culture of axenic fish model to evaluate probiotics and inoculants which possess disease inhibition.

Project Summary

Yellow perch larvae were cultured in high density (30-40 per L; 7.9-10.6 per gal) using live zooplankton for 17 days. The average rate of survival through the entire experimental period was 32.0±7.6% and the swim bladder inflation rate was 35.8±20.6%. The average juvenile weight was 24.5±5.0 mg (0.86±0.18 oz) and average growth rate 29.4±1.6% day-1. Fish were then subjected to treatments with isolated probiotic strains of bacteria and potential pathogenic bacteria isolates. Isolates from adult yellow perch were used to further characterize by heat shock challenge and determine their inhibitory potential against common fish pathogens, *Vibrio anguillarium* and *Aeromonas salmonicida*. Of the eight isolates tested all but three isolates showed inhibition of *Vibrio*, while there appears to be only a weak inhibition to *Aeromonas.*

A feeding experiment was performed with yellow perch juveniles that included dietary treatments with yellow perch isolated probiotic, commercial probiotic, and control. *Flavobacterium columnare* challenge was performed by adding final bacterial density of 108/ml. The final survival rates for treatments did not differ significantly (93- 100%). There were no mortalities during columnaris exposure and no disease symptoms were observed in the following 17 days.

Anticipated Benefits

The proposed studies include comprehensive characterization of the microbiota of the yellow perch digestive tract and surrounding water in production facilities of the North Central Region (NCR). These results will be used to identify cultures of probiotic bacteria that are inhibitory to yellow perch pathogens. It is expected that probiotic strains that can protect yellow perch

juveniles from infection by at least two common pathogens, *Aeromonas* and *Vibrio* species without negative effects on the host fish, will be identified. Therefore, the probiotics identified in this study can potentially contribute to sustainable development of the aquaculture industry and securing an organic produce status for fish.

Project Progress

Objective 1. — Yellow Perch larvae used in 2014 experiments were bred from several 5- 6 year old females from the OSU aquaculture facility and males either from the same source or from Millcreek Perch Farm (Marysville, Ohio). The batch produced for intensive rearing in the OSU aquaculture greenhouse facility originated from egg ribbons that were released and fertilized within the broodstock tank on April 23rd and 25th, 2014.

For Phase I, 50-L (13.2 gal) conical tanks were initially stocked with 1628+ 340 (n=9) larvae/tank. This phase began with the first feeding of larvae at 3 days-post-hatching (dph) and continued throughout the first 10 days of exogenous feeding. The system was equipped with a constant inflow of evaporated sea salt and *Nannochloropsis* algae paste. After 10 days of feeding, 300 larvae were randomly sampled from each tank and moved to the indoor laboratory facility.

Phase II lasted for 7 days fish were reared in nine 60-L cylindrical tanks with constant inflow of water. Temperature remained at 17.2±0.2 ˚C (63 ±32 ˚F) throughout this phase. The rotifers *Brachionus*, a continuous culture maintained at aquaculture lab, and *Artemia* nauplii were hatched from cysts prior to enrichment. During the second phase, fish were initially provided with *Artemia*, then transitioned to Otohime A® diet. The average rate of survival was 32.0±7.6%. Swim bladder inflation rate was 35.8±20.6% at the end of the second phase. The average juvenile weight was 24.5±5.0 mg (0.86±0.18 oz). The results suggest that the growth of yellow perch larvae/juveniles is greater in the EE-enriched groups than the TAG-enriched groups, especially during the first 10 days of exogenous feeding.

Objective 2. — Potential probiotic bacteria were isolated from the intestinal tract of yellow perch collected in OSU aquaculture laboratory. Isolates were challenged by heat shock to further determine their inhibitory potential against common fish pathogens, *Vibrio anguillarium* and *Aeromonas salmonicida*.

To test their direct inhibitory abilities to the two pathogens, we first streak plated on agar with our isolates, heat shocked and cross streaked with the pathogenic species. Of the eight isolates tested all but three isolates showed inhibition of *V. anguillarium* but only weak inhibition to *A. salmonicida*. Once we have determined that our isolates have probiotic potential in-vitro to the selected pathogens, the 16S rRNA gene of the isolates was sequenced. Results indicated that five of the six isolates are strains of *Lactococcus lactis* and one isolate was classified to *Pseudomonas*.

Objective 3.— Preparation of isolates for *in vivo* experiment included isolate V9 and commercial

probiotic 2B. Volume of each culture was adjusted to yield 10^9 cfu/ml and cultures were freeze dried prior to processing into the fish feed. The test consisted of two sets of 12 aquaria that were open (challenge) or semi-recirculating (control). Feeding experiment was performed with yellow perch juveniles (0.08 g). The following dietary treatments were included in the study: commercial diet (control), diet with yellow perch isolated probiotic, diet with commercial probiotic, and yeast and krill based diet. One day before the bacterial challenge fish were divided into 30 fish per tank (designated for the challenge) and the remaining fish were distributed into a parallel system (no challenge).

Objective 4. — Flavobacterium columnare isolation was performed using infected fish (approx. 50% of the external body area infected). Samples were transferred onto a plate with beef extract/agar medium. The colonies were identified (yellowish with not- defined ragged edges). One plate was used to confirm bacterial strain by DNA sequencing method. The bacterial culture from the vial from which the plate had been streaked was used to inoculate additional cultures. In order to carry out columnaris challenge, bacterial culture was added to each tank to provide final bacterial density of 10^8 /ml. The desired colonies were found in the challenged group but not in the uninfected group. The density of bacterial colonies in challenged tanks was estimated as $8.7*10^5$ CFU/ml. The results at the completion of the feeding experiment indicated the largest weight was observed in fish that were fed the control diet (0.57±0.02 followed by probiotic supplemented groups, 0.51±0.13, 0.42±0.03, and 0.23±0.02 g in experimental diet. The final survival rates for treatments after the challenge were 100, 98, 96, and 93%, respectively. There were no mortalities during the 24-h columnaris incubation period. No disease symptoms were observed due to introduction of columnaris bacteria. To date, construction and refinement of the axenic fish model needs to be completed for this objective.

Target Audiences

Fish culture operations in the North Central Region (NCR) have all experienced disease outbreaks on occasion, resulting in significant monetary loss. Good husbandry practices can significantly reduce but not eliminate such outbreaks. Given that most aquaculture in the NCR occurs in ponds, administering chemotherapeutic drugs is not economically feasible because the large amount of water in individual ponds precludes treating the water and individual fish from many NCR species often cease or reduce feeding once infected by a pathogen. The industry has long recognized that feeding a nutrient complete diet is a good husbandry practice and that inclusion of probiotics that increase resistance to common pathogens would enhance the effectiveness of such a diet. A cost- effective reduction in fish losses will increase the economic viability of all culture operations within NCR.

Outreach Overview

Oral presentation to the joint annual meeting of the Ohio and Michigan Aquaculture Associations in Toledo, February 22, 2014.

Deliverables (Outputs)

Nothing to report

Outcomes/Impacts
Nothing to report

Impacts Summary
Relevance. — Yellow perch are often stocked at high densities under environmentally stressed conditions that often result in increased number of diseases.
Response. — The proposed studies include comprehensive characterization of the microbiota of the yellow perch digestive tract and surrounding water in production facilities of the North Central Region (NCR). These results will be used to identify cultures of probiotic bacteria that are inhibitory to yellow perch pathogens. It is expected that probiotic strains that can protect yellow perch juveniles from infection by at least two common pathogens, *Aeromonas* and *Vibrio* species without negative effects on the host fish, will be identified.
Results. — Potential probiotic bacteria were isolated from the intestinal tract of yellow perch collected in OSU aquaculture laboratory.
Recap. — The probiotics identified in this study can potentially contribute to sustainable development of the aquaculture industry and securing an organic produce status for fish.

Publications, Manuscripts, Workshops, and Conferences
See the Appendix for a cumulative output for all NCRAC-Funded Aquaculture Drugs activities.

Project Title: Economic Impact Assessment [Termination Report]
Key Word(s): Economics/Marketing
Total Funds Committed: $18,810
Initial Project Schedule: September 1, 2012 to August 31, 2014
Current Project Year: September 1, 2013 to August 31, 2014
Participants: Steven G. Deller, University of Wisconsin Extension, Wisconsin; Christopher F. Hartleb, University of Wisconsin-Stevens Point, Wisconsin; Laura G. Tiu, The Ohio State University, Ohio
Extension Liaison: James A. Held, University of Wisconsin Extension, Wisconsin
Reason for Termination: Project objectives completed and funds have been terminated per multiple participants' resignations.

Project Objectives

1. Characterize the aquaculture industry throughout the NCR (species, systems, purpose, size, sales, jobs, etc.).
2. Determine the direct, indirect and induced contributions of the aquaculture industry to regional and state – by - state economies.

Project Summary

The aquaculture industry in the NCR suffers from a lack of clearly documented information that describes the industry (e.g., species, systems, purpose, size, sales, jobs, etc.) and its economic value to the region. This information is critical to assist NCR producers to inform and leverage the support of political, regulatory and educational decision-makers on issues that impact aquaculture production and expansion of the industry.

Technical Summary and Analysis

Using the 2013 Census of Aquaculture we explore the contribution of aquaculture to the North Central Region (NCR) economy. In 2013 there were 336 aquacultural enterprises with total sales of $36.7 million, for average sales of $109,300 per firm. Using input-output analysis to construct regional economic models of the NCR states we find that this level of activity created 520 jobs, $16.8 million in labor income (wages, salaries and proprietor income), $36.5 million in total income and $71.2 million in industrial sales or revenue. This economic activity generates $2.6 million in state and local government revenues and over $3.8 million in federal government revenues. The level of economic impact of aquaculture varies significantly across the NCR states with the smallest impact being in Kansas and the largest in Missouri.

Principal Accomplishments

Objectives 1 & 2.— According to the latest US USDA Census of Aquaculture (2013) the North Central Region (NCR) states there are 336 aquacultural enterprises with total sales of $36.7 million, for average sales of $109,300 per firm; North Dakota was not included in this study due to lack of identified aquaculture operations. There is significant variation in aquacultural activities across the NCR states. Kansas has the lowest level of activity with only four firms and $571,600 in sales, but per firm sales is greater than the regional average. Wisconsin has the most aquaculture firms at 80 but has one of the lowest per firm sales in the Midwest. It is clear that

there is significant heterogeneity in the scale of aquaculture across the NCR states. Indeed, Indiana has only seven firms but the average sales per firm is the highest in the region at $311,800.

Impacts

In 2013 there were 336 aquacultural enterprises with total sales of $36.7 million, for average sales of $109,300 per firm. In this study we find this aquaculture supports 520 jobs and $71.19 million in industrial sales in addition to $16.8 million in labor related income. The North Central Regional state with the largest employment impact is Missouri with 216 jobs, Wisconsin with 128 jobs and Ohio with 104 to only seven jobs in Kansas. The economic activity associated with aquaculture also generated tax revenues that flow to state and local governments in the study region ($2.65 million) as well as the federal government ($3.83 million).

Recommended Follow-Up Activities

Future economics projects should be considered as new USDA aquaculture census are developed as this information is critical to understanding the economic impacts of the regional aquaculture industry.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Economics/Marketing activities.

Technical Update

Table 1: Aquaculture Activity in the Midwest

2013 Census of Aquaculture	Sales ($1,000)	Number of Establishments	Sales per Establishment ($1,000)
Illinois	$2,861.3	23	$124.4
Indiana	$2,182.8	7	$311.8
Iowa	$2,811.1	31	$90.7
Kansas	$571.6	4	$142.9
Michigan	$1,529.2	32	$47.8
Minnesota	$5,615.6	35	$160.4
Missouri	$7,437.3	35	$212.5
Nebraska	$2,405.2	21	$114.5
Ohio	$4,757.9	61	$78.0
South Dakota	$1,233.2	7	$176.2
Wisconsin	$5,303.4	80	$66.3
Region as a Whole	$36,708.6	336	$109.3

Table 2: Detailed Impact Analysis Aquaculture and the North Central Region

	Employment	Labor Income (000$)	Total Income (000$)	Industry Revenue/Sales (000$)
Direct	319	$7,702.9	$19,934.9	$36,441.2
Indirect	109	$4,865.2	$9,139.9	$21,929.8
Induced	93	$4,283.4	$7,422.2	$12,817.7
Total	520	$16,851.6	$36,497.1	$71,188.7
Agriculture	380	$9,806.8	$23,243.3	$44,711.1
Mining	1	$24.8	$51.2	$153.8
Construction	4	$227.1	$287.6	$484.4
Manufacturing	8	$665.9	$1,913.1	$8,314.3
Trans. Ins. Pub. Utilities	9	$626.1	$1,134.9	$2,070.9
Trade (Retail & Wholesale)	24	$1,133.8	$1,854.3	$2,683.6
Services	93	$4,226.0	$7,860.2	$12,492.7
Government	2	$141.2	$152.5	$277.8

Table 3: Tax Revenue Generated (000$)

Sales Taxes	$954.7
Income Taxes	$359.0
Property Taxes	$937.2
Other	$401.3
Total St & Loc Revenue	$2,652.2
Corp Profit Taxes	$1,140.7
Income Taxes	$1,100.5
Other	$1,594.1
Total Federal Revenue	$3,835.4

Table 4: Economic Contrubutions of Aquaculture ($1,000)

	Sales (2013 Census)	Number of Farms	Sales per Farm	Employment	Labor Income (000$)	Total Income (000$)	Industry Revenue/Sales (000$)
						Economic Impacts	
Illinois	$2,861.3	23	$124.4	32	$1,259.0	$2,552.7	$4,717.2
Indiana	$2,182.8	7	$311.8	32	$756.6	$1,801.2	$3,389.4
Iowa	$2,811.1	31	$90.7	24	$937.9	$2,292.9	$4,368.8
Kansas	$571.6	4	$142.9	7	$215.9	$454.5	$858.2
Michigan	$1,529.2	32	$47.8	36	$684.7	$1,295.6	$2,406.0
Minnesota	$5,615.6	35	$160.4	64	$1,924.6	$4,851.7	$9,241.2
Missouri	$7,437.3	35	$212.5	216	$2,503.1	$6,145.5	$11,527.9
Nebraska	$2,405.2	21	$114.5	18	$854.2	$1,902.8	$3,621.8
Ohio	$4,757.9	61	$78.0	104	$2,025.7	$4,108.0	$7,782.1
South Dakota	$1,233.2	7	$176.2	12	$543.1	$982.1	$1,852.7
Wisconsin	$5,303.4	80	$66.3	128	$2,496.5	$4,508.0	$8,422.5
Region as a Whole	$36,708.6	336	$109.3	520	$16,851.6	$36,497.1	$71,188.7

Project Title: Extension [Progress Report]
Key Word(S): Extension
Total Funds Committed: $1,176,685
Initial Project Schedule: May 1, 1989 to August 31, 2015
Current Project Year: September 1, 2014 to August 31, 2015
Participants: Dennis E. Bauer, University of Nebraska-Lincoln, Nebraska; Mark E. Clark, North Dakota State University, North Dakota; James A. Held, University of Wisconsin-Stevens Point, Wisconsin; Charles E. Hicks, Lincoln University, Missouri; Paul Hitchens, Southern IL University – Carbondale, Illinois; Ronald E. Kinnunen, Michigan State University, Michigan; Charles D. Lee, Kansas State University, Kansas; Allen Pattillo, Iowa State University, Iowa; Burton F. Pflueger, South Dakota State University, South Dakota; Nicholas Phelps, University of Minnesota, Minnesota; Kwamena K. Quagrainie, Purdue University, Illinois/Indiana; Laura G.Tiu, Ohio State University, Ohio; Christopher Weeks, Michigan State University, Michigan
Industry Liaison: Mark Willows, Binford Eagle Fisheries, North Dakota

Project Objectives

1. Strengthen linkages between North Central Regional Aquaculture Center (NCRAC) Research and Extension Work Groups.
2. Enhance the NCRAC extension network for aquaculture information transfer.
3. Develop and implement aquaculture educational programs for the North Central Region)

Project Summary

The existing aquaculture industry members need relevant information on new techniques and technologies in aquaculture, as well as updated information related to changing state and federal regulations. Increasingly, a large number of individuals are interested in aquaculture as a means of agriculture diversification or urban development. The NCRAC Extension Work Group meets these diverse client needs through on-site advice, publications, and specialized workshops. As the industry matures, the advisory service needs will shift toward more specialized and advanced knowledge than is currently provided at general introductory conferences and events. Entrepreneurs and prospective aquaculturists often require an enormous amount of time to educate and can benefit from the availability of the electronic media.

Anticipated Benefits

The NCRAC Extension Work Group will continue and expand its efforts to promote and advance commercial aquaculture in a responsible fashion through its organized education/training outreach programs and through educating the public on the health benefits of commercially raised fish. The primary benefits are: increased public awareness through publications, short courses, and conferences regarding the potential of aquaculture as a viable agricultural enterprise in the NCR; technology transfer; improved lines of communication between interstate aquaculture extension specialists and associated industry contacts; and an enhanced legal and socioeconomic atmosphere for aquaculture in the NCR. The development of aquaculture education programs for the NCR has provided “hands- on “opportunities for prospective and

experienced producers.

Approximately 6,000 individuals have attended workshops or conferences organized and delivered by the NCRAC Extension Work Group. Clientele attending regional workshops have gained information related to aquaculture development strategies in other areas of the country and acquired information which was of direct use to their own enterprises. Education programs also created situations where problems encountered by producers were expressed to extension personnel who later relayed them to researchers at NCRAC work group meetings for possible solutions through the research effort.

Project Progress

Objective 1. — Aquaculture Extension

Work Group members have:

- Served as an extension liaison, if not an active researcher, for every NCRAC-funded project;
- Assisted in developing, writing, and editing several culture manuals as well as fact sheets, book chapters, and videos based on NCRAC-funded research;
- Assisted with the planning, promotion, and implementation of taxa-specific workshops held throughout the region;
- Participated as Steering Committee members for public forums related to revision of the National Aquaculture Development Plan and the four past National Aquaculture Extension Workshops/Conferences;
- Served as a non-funded collaborator on the Regional Aquaculture Extension Specialist; and
- Met with industry representatives and university researchers involved with aquaculture to discuss how the aquaculture industry could grow in the NCR.

Objective 2.— Networking of specialists and Cooperative Extension Service (CES)- designated contacts has maximized the efficiency of education programs and minimized duplication. Individual state extension contacts often respond to 120+ annual calls from outside their respective state as well as interacting with colleagues with mutual concerns related to developing aquaculture activities. This extension network is critical to being able to match specific aquaculture questions with the best source of information. To better illustrate individual state extension specialist's role in regional and state extension programs, the following are a partial list.

For instance, Lee has continued to assist the Kansas Aquaculture Association by developing, printing and distributing the Kansas Aquaculture Association Directory. In a similar fashion, Bauer distributed NCRAC information to the Nebraska aquaculture industry; and in North Dakota, Clark developed an updated list of state producers for submission to the NCRAC Publications Office as well as worked with state public agency personnel concerning

state/federal regulations for North Dakota producers. Hicks developed three aquaculture factsheets on freshwater prawn and bluegill production while also conducting In-Pond-Raceways tests with cooperating farmers. Regional extension cooperators for the eXtension project (including the 2012 eXtension Aquaculture Virtual Workshop), including Jim Held, Allen Pattillo, Ron Kinnunen, and Laura Tiu who have presented on a variety of topics including aquaponics, species- specific culture (bluegill, freshwater prawn, yellow perch, and walleye), recirculating systems, prospective considerations of aquaculture operations, and HACCP training. Pattillo developed a suite of 13 downloadable fact sheets, standard operating procedures, and example data collection sheets for fish feeding, water quality, fish health, and calculation rates for RAS. Regional extension cooperators for the eXtension project provided hands-on workshops and online training for producers (including the 2013 eXtension Aquaculture Virtual Workshop), including Jim Held, Allen Pattillo, Ron Kinnunen, and Laura Tiu who have presented on a variety of topics including aquaponics, species-specific culture (bluegill, freshwater prawn, yellow perch, and walleye), recirculating systems, aquaponic system design and management, prospective considerations of aquaculture operations, and HACCP training.

Objective 3. — A number of workshops, conferences, symposia, videos, field-site visits, hands-on training sessions, and other educational programs have been developed and implemented (see the Appendix for a listing of many of these activities). Through these workshops, critical issues in the private aquaculture industry have been identified, e.g., market availability, economic returns, and regulatory concerns. NCRAC Extension contacts have served as editors for regional aquaculture newsletters as well as in-state aquaculture association newsletters; served on state aquaculture advisory councils and state aquaculture task forces; and assisted in the planning and implementation of state aquaculture association meetings. In addition to the previously mentioned areas, NCRAC Extension contacts have been instrumental in fostering the continued growth of the aquaculture industry in the region through a variety of activities and many have worked with industry and governmental representatives to produce state aquaculture plans and improved governmental regulations. An AIS-HACCP plan has also been developed by Kinnunen and Phelps to address the growing concern of biosecurity, particularly in regard to diseases such as viral hemorrhagic septicemia (VHS). Kinnunen and Phelps have also taught other members of the NCR aquaculture extension community about their AIS-HACCP program, in essence, they've "trained the trainers" and all AIS-HACCP materials are available at www.seagrant.umn.edu/ais/haccp. More recently, Kinnunen participated in the Michigan DNR Lake Superior Citizen Advisory Committee meeting and presented how the AIS-HACCP program can be used to prevent the spread of aquatic invasive species in baitfish. The effectiveness of the AIS-HACCP is reflected in the fact that aquatic invasive species have not been identified in the baitfish trade according to surveys conducted by Michigan DNR staff. Nick Phelps (University of Minnesota Veterinary Diagnostic Lab) and Kinnunen also conducted multiple Aquaculture Biosecurity/AIS-HACCP Workshops.

Outreach Overview
Enhancing state-wide and regional communication and training among those in the aquaculture industry is imperative for continued growth of aquaculture in the Midwest. Aquaculture Extension Specialists are important to the distribution of aquaculture extension related materials, providing research-based information to the farmers who will use it. Additionally, promoting networking between public institutions and private aquaculturists helps enhance the transfer of aquaculture information and technology.

The workshops were mainly hands-on, which enabled participants to acquire knowledge and skills in indoor recirculating aquaculture systems. Some workshop participants have started aquaculture operations after attending the workshops. Additional services include on-line educational materials, workshops, business planning assistance, facility tours and production training.

Target Audiences
Current and prospective fish farmers.

Deliverables (Outputs)
The following reflects a partial list of the extension activities performed over the project period. Quagrainie (Indiana) developed extension publications covering shrimp biofloc production and hybrid striped bass cage aquaculture and economics. Pattillo (Iowa) had 24 events in 2014, reaching nearly 2,300 people. He delivered 13 presentations, eight workshops, four conferences, eight field days/farm tours/especial events, five news releases, five videos, and 13 extension publications. Highlights include the Bio-dome aquaponics exhibit at Reiman Gardens (Iowa State University), which had over 41,000 visitors in 2014.

Muthukumarappan (South Dakota) replaced Burton Pfleuger during this project. Muthu participated in two high impact conferences where he presented on the use of oil seeds like canola, soybean, carinata and camelina meals to the manufacture of high-protein aquafeeds.

Hicks (Missouri) conducted two projects involving 1) Demonstration of in-pond-raceways for the production of fish in existing private ponds and lakes and 2) Production of Guide sheets to transfer research information to stakeholders. These targeted low resource farmers involved and those interested in aquaculture. Results were extended to the audiences by providing presentations at local aquaculture meetings, schools, field-days, and Youtube videos. At least two farmers initiated aquaculture enterprises based on information transferred from this research. Four extension publications were developed on the topics of pond construction, hybrid sunfish, fish stocking, and aquatic plant management.

Kinnunen (Michigan) attended and presented at eight meetings on AIS-HACCP/Aquaculture Biosecurity for the aquaculture and baitfish industry and collaborated with Weeks (Michigan) and Phelps (Minnesota) on a funded grant proposal on "Towards AIS Free Certification in Aquaculture and Baitfish Industries. Kinnunen coordinated a three day Seafood HACCP Training with 21 attendees from state and tribal fishermen, aquaculture producers, fish processors, and representatives from feed companies. Kinnunen has played a major role in the Michigan Sea Grant Aquaculture Integrated Assessment Project dealing with for the potential of net pen culture in the Great Lakes.

Tiu (Ohio) directed the Aquaculture Technology Transfer (AT2) program, which provides information and training for the aquaculture industry. Services include on-line educational materials, workshops, business planning assistance, facility tours and production training. A close working relationship with the Ohio Aquaculture Association, the producer association in Ohio helps drive the growth and success of aquaculture in the state. Activities for 2014 include continued expansion of the Aqua-Ohio and other List serves that provide timely dissemination of aquaculture related news and resources to over 790 clients throughout the region, nationally, and internationally. The Ohio Aquaculture Association (OAA) collaborated with NCRAC to host the 2014 NCR Aquaculture Conference in Toledo, OH; reaching over 250 fish farmers and industry experts. Presentations from the conference are here: http://southcenters.osu.edu/node/1470. Workshops and farm tours were held for Aquaponics, RAS, and an Aquaculture Bus Tour of Ohio Farms.

Outcomes/Impacts

All extension programming was developed around the concept of increasing producer knowledge to increase production efficiency and profitability. NCRAC extension programs reached thousands of individuals during this project. For example, in Missouri, Hicks documented the startup of two aquaculture business after attending his extension programs. However, because of the nature of the aquaculture industry and the variety of information transfer methodologies employed, it is difficult to capture outcomes and impacts. The continuing benefit of web-based resources is un-measurable. Because of the inherent risky and laborious nature of aquaculture, a major function of an extension specialist is to provide the harsh realities of aquaculture business, which may lead to the prevention of money lost by startup producers - this benefit is un-measurable.

Impacts Summary

Relevance. — Fish farmers require some basic extension services including responding to various questions relating to fish production. Extension activities would include providing resources relating to addressing issues such as poor water quality, diseases, low oxygen levels, water temperature, and feeding strategies. Some prospective fish farmers need farm visits to assist with hands-on experiential learning on various fish production issues.

Response. — Workshops and on-line materials developed.

Results. — Participants to acquire knowledge and skills in indoor recirculating aquaculture systems.

Recap. — In response to industry need, workshops have been identified throughout the region to address industry issues.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-funded Extension activities.

Project Title: Regional Aquaculture Extension Specialist (RAES) [Progress Report]
Key Word: Extension
Total Funds Committed: $205,165
Initial Project Schedule: September 1, 2013 to August 31, 2015
Current Project Year: September 1, 2014 to August 31, 2015
Participant(s): Christopher Weeks, Michigan State University, Michigan
Extension Liaison: K. Quagrainnie, Purdue University, Indiana
Industry Liaison: William Lynch, Mill Creek Perch Farms, Marysville, Ohio

Project Objectives

1. Continue RAES support to the NCR aquaculture community through ongoing activities in areas of services, leadership, assessing and addressing industry needs, and information transfer
2. Develop and implement strategies to address and promote aquaculture sustainability in the NCR.
3. Develop and strengthen partnerships from within the NCR and outside the region among regulatory agencies, industry, academia, and other relevant entities to foster open, meaningful dialog on critical issues and build support for the NCR aquaculture industry.
4. Coordinate efforts for seeking non-NCRAC support for NCR aquaculture development.

Project Summary

Extension has been identified as a top priority by the NCRAC Industry Advisory Committee and the NCR aquaculture community. The Regional Aquaculture Extension Specialist (RAES) project was initiated in 2008 under an overarching goal of advancing commercial aquaculture in the region. RAES initial objectives were to provide leadership and enhance information transfer to the aquaculture industry in the North Central Region (NCR). The RAES project has been rated high in subsequent years by NCRAC members and has been re-funded for continuation twice under expanded objectives. This has been advantageous in that work plans have been able to build upon previous project activities and outcomes. Through the RAES project, regional coordination has helped improve liaison services, project coordination, strategic planning, program facilitation, partnership building, outreach, and information dissemination to the aquaculture community at a time when aquaculture extension FTE's in individual states have been dropping. Currently there are less than 5 FTE's in aquaculture extension in the NCR; however, 1-2 more are expected in the near future. On July 1, 2014, PI Weeks moved from a full time appointment as RAES, to a shared 50% appointment; remaining appointment is with Michigan State University Extension.

Anticipated Benefits

- Information transfer to the aquaculture community via list serve, websites, and state association events and other direct contact methods.
- Continued updates on the NCRAC regulation website.
- An industry voice on state, regional and national regulatory issues such as AIS.
- A better understanding on ability to develop the current AIS HACCP program into a recognized certification or verification program.
- Strengthened partnerships for NCR aquaculture development.

- Submission of at least one grant proposal for NCR industry support in 2014-2015.

Project Progress
Activities in areas of liaison services, leadership, assessing and addressing industry needs, and information transfer are noted in the following activities.

Objective 1. — Full update to the NCRAC Regulation website "State Importation and Transportation Requirements for Cultured Aquatic Animals". The PI served on planning steering committees for the 2014 NCR Aquaculture Conference in Toledo, OH, and 2015 Michigan Seafood Summit in East Lansing, MI. Between 9/1/13 and 8/31/15 the PI provided 11 presentations for stakeholders, public agencies, association meetings and conferences including key note addresses in OH and IA. The PI supported speakers to present at the NCR Aquaculture Conference. Current and/or important information was disseminated to stakeholders by the RAES via phone, emails, NCR fish culture list serve and eXtension Ask-an-Expert. Liaison activities included industry representation at public meetings likely to impact aquaculture and baitfish industry sectors (e.g., Great Lakes Panel for ANS), identifying contacts for industry consultation, and leading case by case discussions on regulatory issues for NCR producers. PI took lead role in the 2014 NCR aquaculture needs survey. This survey examined impediments impacting NCR aquaculture and where priorities should be placed for improvement.

Objective 2. — PI voiced industry concerns and interests to the Policy Committee of the Great Lakes Panel for ANS at bi-annual meetings. PI and R. Kinnunen conducted two AIS HACCP workshops for MI DNR and MI Bait Dealers Association. Weeks, Kinnunen (MI Sea Grant) and Phelps (UMN) were awarded $80,000 grant through state of Michigan to explore expanding the current AIS HACCP program into a recognized verification/certification program (ongoing). Case studies are planned for states of MI and MN in 2016. PI collaborated with Ron Johnson, Nathan Stone (National ANS Task Force), and Mike Freeze (NAA President) on current national ANS issues. Pertinent and topical information was disseminated to aquaculture industry, supporters, and other subscribers to the NCR list serve.

Objective 3. — The RAES represented industry interests at various private and public forums and has standing appointments to the Committee for the Right to Farm Generally Accepted Agriculture and Management Practices, Michigan Commission of Agriculture; Great Lakes Panel on Aquatic Nuisance Species; Aquaculture in Michigan (AIM), NSF International Global Food Division Advisory Council. In 2014 the RAES was voted into the NCRAC Board as an ex-officio member, working closely with NCRAC Director and others on issues such as improving NCRAC's project selection protocols and updating the NCRAC Strategic Plan. The RAES also helped to build and strengthen partnerships with a number of other organizations including, Indiana and Michigan Soybean Associations, Soy Aquaculture Alliance, Coalition for U.S. Seafood Production, National Institute for Sustainable Aquaculture, initiative for Ohio Seafood co-op, and private agricultural business startup groups such as Originz. In addition, the RAES continued working with nonprofit groups

including Aquaculture Research Corporation on Great Lakes open water aquaculture issues, and the Nature Conservancy on aquatic invasive species issues.

Objective 4. — From 9/1/13 to 10/31/15 the RAES established teams of regional researchers and extension personnel and submitted two proposals as PI to non-NCRAC funding sources. Two additional proposals were submitted to outside granting agencies with Weeks as co-PI.

Target Audiences
The RAES ultimately serves the aquaculture industry in the North Central Region. However, goals and objectives require working with stakeholders from across a wide background and include the general public, state, federal and tribal agencies, legislators, NGOs, industry and social development groups, and environmental protection groups.

Outreach Overview
In 2014 the NCRAC Regulation website received 4,689 page views. Through great combined effort, the 2014 North Central Aquaculture Conference was considered a great success with over 170 in attendance, providing an average overall evaluation score of 3.3 out of 4.0. Presentations received an average score of 3.6. There were 125 individuals in attendance at the 2015 Michigan Seafood Summit. Thirty individuals were trained in AIS HACCP, and two courses presented received good to excellent reviews. Project PI provided over 200 information posts per year by PI to the NCR Fish Culture List serve containing 140 members. These posts included relays of posts from other information outlets such as AquaContacts, and contained pertinent and important information such as summaries of Federal Register postings for new laws, economic and market issues, topical news, etc. Direct and personal communications for the RAES are estimated at over 500 individuals per year. Project PI facilitated discussion for producers in Ohio and Wisconsin with state of Michigan regulatory agencies leading to improved working environments for those producers. North Central Region Aquaculture Needs Survey results were disseminated to NCRAC members and used for 2014 and 2015 program planning activities. In 2014, a Strategic Plan for a Thriving & Sustainable Michigan Aquaculture was completed and adopted by the Michigan Aquaculture Association. The final report is available online on the Michigan Sea Grant Website.

Deliverables (Outputs)
Over the continuation period from 2013 the RAES provided the following deliverables:
Objective 1:

- Full update to NCRAC regulation website.
- Support in planning and facilitation of the 2014 NCR Aquaculture Conference in Toledo, OH, and 2015 Michigan Seafood Summit in East Lansing, Michigan.
- Attendance at over 20 meetings/conferences
- Provided 11 presentations for stakeholders, public agencies, association meetings and conferences including key note addresses in Ohio and Iowa.

Administered and obtained results from the 2014 NCR Aquaculture Survey which were used in NCRAC strategic planning activities.

- Support resulting in update to Objective 1 of the NCRAC Strategic Plan • Development of the Michigan Aquaculture Strategic Plan.
- GIS model results examining temperature current and depth for potential siting of netpen facilities in the great Lakes.

Objective 2:

• Two AIS HACCP workshops for MI DNR and MI Bait Dealers Association.
• Two meetings in Minnesota at MNDNR and Oswald Fisheries.

Objective 3:

- Updated Generally Accepted Agriculture and Management Practices (GAAMPS), for Michigan.
- Provided industry representation at biannual Great Lakes Panel for Aquatic Nuisance Species and annual meetings for the NSF International Global Food Division Advisory Council.
- Developed working relationships with: - Kent State University, Tuscarawas Campus, to explore Cooperative Opportunities in the Ohio Fish Farming Industry (ongoing) - Jackson College, Jackson, Michigan for planning development of an aquaculture curriculum (ongoing).
- NCR industry representation at the NIAA's National Roundtable for Sustainable Aquaculture (ongoing).

Objective 4:

Submitted grant applications over the project period were as follow: USDA AFRI; A Practical Approach to Rapid Aquaculture Development across Midwestern US Rural Communities; $499,408; status – not funded. State of Michigan (through GLRI); Towards AIS Free Certification in Aquaculture and Baitfish Industries; $79,985; status – awarded. NOAA Sea Grant Aquaculture Extension 2015: Assisting the State of Michigan and Michigan Industries in Developing and Permitting More Sustainable Aquaculture Systems; $451,611; status – awarded. NSF ATE grant; Jackson College Aquaculture Project: Meeting the Nation's Growing Demand for Locally Sourced Seafood; $200,000; status – submitted.

Outcomes/Impacts

Due to complexities associated with advancing finfish aquaculture in the US, direct measurable impacts from RAES activities are difficult to assess. In the state of Michigan, two new NPDES permits were issued bringing the total number to three. A number of indoor startup facilities have arisen across the region although economic viability of NCR indoor recirculating aquaculture systems (RAS) and aquaponics remains questionable. The recent closing of Bell Aquaculture is a prime example. In order to identify needs and priorities, the RAES along with IAC Chair and NCRAC Director developed and administered the 2014 NCR Aquaculture Survey. Results have been disseminated to NCRAC members and have

been used and referenced extensively for strategic planning activities (ongoing).

Impacts Summary

Relevance. — According to the USDA 2013 Census, aquaculture production in the NCR is down from 2005 while value has increased. Extension FTE's across the region have been dropping. Regulations continue to be the primary industry concern limiting expansion, and it appears the public has a strong perception that aquaculture expansion must be through RAS for a variety of reasons.

Response. — 2014 NCR Survey results indicate that regulations are considered the largest impediment to industry expansion. Annual updates to the NCRAC regulation website and RAES liaison activities have been noted as helpful. The RAES and others in the NCRAC community continue to try to identify economic related problems with profitability of indoor systems (ongoing).

Results. — The 2014 NCR aquaculture survey received a good response rate (37%), suggesting respondents thought aquaculture development in the NCR is important to them. Subsequent NCRAC planning activities have brought forward initiatives for changes in NCRAC program planning including priority selection protocols and identification of extension as a leading priority by the IAC. According to producers and interstate transporters confusion regarding the extensive array of regulations across states has been helped by RAES activities, and in some cases communications between producers and state agencies is apparent. Since the release of the Michigan Aquaculture Strategic Plan there has been a flurry of activity in Michigan regarding development of netpen aquaculture systems in the Great Lakes. Two proposals were subsequently submitted to the state for commercial rainbow trout production facilities. In response the state of Michigan commissioned reports on environmental and economic concerns and issues, and on regulatory framework regarding netpen aquaculture in Michigan. These reports are available for review at the Michigan Department of Agriculture Aquaculture website.

Recap. — With the exception of Bell Aquaculture, aquaculture production in the NCR appears to have come to a stable point and could be beginning to increase; however, much work is yet remains in the area of indoor system profitability and overcoming negative impacts from regulations.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-funded Extension activities.

Project Title: Establishing Largemouth Bass Strains for Rapid Growth to 1.5 Pounds in the North Central Region [Progress Report]
Key Word(s): Largemouth Bass
Total Funds Committed: $155,000
Initial Project Schedule: September 1, 2014 to August 31, 2016
Current Project Year: September 1, 2014 to August 31, 2015
Participants: Brian Small, Southern Illinois University- Carbondale; Han-Ping Wang, The Ohio State University, Ohio; D. Glover, The Ohio State University, Ohio
Extension Liaison: Paul Hitchens, Southern Illinois University, Carbondale

Project Objectives

1. Identify the best genetically distinct largemouth bass populations for fast growth in the NCR.
2. Conduct a meta-analysis using all appropriate data for largemouth bass from both published and non-published sources to identify at minimum three populations of LMB with the potential to exhibit rapid growth to target weight in the NCR.
3. Evaluate the identified populations at two or more latitudes in the NCR to identify the optimal source population

Deliverables

1. Publication of results in journal articles(s).
2. Extension products, including a selection mix.

Project Summary

Largemouth bass (LMB) is an important aquaculture species. Interest in improving commercial culture efficiency has grown due to the great demand and high value compared to other cultured species. While this fish has been extensively investigated for management of the fisheries and hatchery production, little research has been conducted to maximize growth for commercial foodfish production. A NCRAC Priority is to increase the efficiency of LMB growth to market size through means beyond dietary modification. One impediment beyond nutritional insufficiency is the rearing of LMB stocks with little to no domestication or selective breeding for efficient production. Therefore, strain evaluation and identification of the best largemouth bass populations for fast growth would result in an immediate impact on the economic return of many small aquaculture operations in the North Central Region (NCR). At the completion of this project, anticipated outcomes include a description of LMB genetic diversity among commercial and public stocks available to NCR, the development of selection markers and a matrix for fast growing LMB, and the identification of fast growing populations verified in production systems.

Anticipated Benefits

The great demand for largemouth bass and their high selling price and growth rate (compared to other cultured species) have raised interest in their commercial culture. Differential performance of genetic strains of largemouth bass is an important management consideration for both recreational fisheries and aquaculture. Therefore, strain evaluation and identification of the best genetically

distinct largemouth bass populations for fast growth and the optimal source population would result in an immediate impact on the economic return of many small aquaculture operations in the North Central region. A NCRAC Priority is to increase the efficiency of LMB growth to 1–1.5 pounds through means beyond dietary modification. At the completion of this project, we expect to identify the best populations of LMB with the potential to exhibit rapid growth to target weight for the NCR aquaculture industry.

Furthermore, a description of diversity among commercial and public stocks available to NCR producers will be available. At present there is little consensus regarding the stocks currently produced, with opinions that all the LMB produced are of similar genetic background, coming primarily from a single fingerling producer, to the opinion that many producers have already selected for fast growth on their own. If stock improvement is to be made, a thorough investigation of the genetics and the management must first be assessed. Objectives 1 and 2 will address these issues. Specifically, Objective 2 will take the growth information obtained from the literature, surveys, and public databases to predict stocks of fast growing fish for the NCR. As a result, a selection matrix will be made available to commercial producers. The third objective will validate the results of Objective 1, used for selecting fast growing stocks, by conducting production studies during the first year of growth. Although this project does not provide funding beyond year 2, it is anticipated that the PIs will be able to find funding to continue the production studies to market weight and ultimately present conclusive evidence to the NCR producer. Regardless, workshop materials will be developed to discriminate the results and train producers how to use the genetic data and selection matrix for their own breeding programs. Results will also be published as factsheets and research papers.

Project Progress

Objective 1. — In this first year, much work has been completed on objective 1. The Ohio State University (OSU) researchers investigated the genetic structure of largemouth bass from 20 wild populations and five cultured stocks across the United States and China using eight microsatellite loci, which are standard genetic markers for population genetic analysis. Our major findings are as follows: (1) Allelic richness was lower among cultured populations than among wild populations; (2) Effective population size in hatcheries could promote high levels of genetic variation among individuals and minimize loss of genetic diversity; (3) The majority of largemouth bass populations had a significant heterozygosity excess, which is likely to indicate a previous population bottleneck; and (4) The phylogeny based on eight microsatellites revealed a clear distinction between northern and southern populations, although samples from 25 populations were different (Fig. 1).

The information provides a valuable basis for development of aquaculture genetic breeding programs in largemouth bass. These results suggest that (1) collecting wild populations for developing broodstock would be better for production of fast growing population for aquaculture; (2) if hatchery populations would be used for developing broodstock for aquaculture, large effective population size will be needed to promote high levels of genetic variation and minimize loss of genetic diversity; and (3) it would be better to use genetically

diversified northern LMB populations for development of broodstock.

Southern Illinois University Carbondale (SIUC) collected DNA from 30 LMB populations across the NCR in year 1. DNA from each fish was sent to OSU for analysis of genetic diversity, to be determined in year 2 and added to the results discussed above. Analysis of putative genetic growth markers associated with the IGF-I (Table 1) and IGF-II genes was conducted on these samples at SIUC.

In year 2, the 20 populations from OSU will also be analyzed for these putative growth markers. Unique alleles for these genes were identified NRC fish and share some similarities to those published for increased growth in Chinese LMB populations (Li et al. 2009, 2012). We also identified a unique allele for IGF-II in some of the populations. Based on our results and those of Li et al. (2009, 20012), four LMB stocks were selected for a juvenile growth study to verify predicted growth associations. The top and bottom 5% of within each stock will be genotyped for correlation to growth performance. These results will factor into stock selection for Objective 3.

Objective 2. — Objective was completed in year 1 by collecting largemouth bass weight- and length-at-age data from LMB populations throughout the NCR. Meta-analysis results by state suggested Kansas had the fastest growing LMB with an estimated time to market size of 1.05 years. However, Kansas had data from only one reservoir. States with larger data sets were ranked (years to reach market size) as follows: Illinois (1.38), Iowa (1.56), South Dakota (1.81), Wisconsin (2.22), Minnesota (2.40), Ohio (2.53), and Nebraska (4.19). Remaining NCR states either did not send data or it was insufficient for use with the statistical model, as many hatcheries and fish farms do not collect and keep records of data or were unwilling to share information.

Objective 3. — Activities will be conducted in year 2 following completion of objective 1 genetic diversity and validation of growth markers. We will collect broodfish from three to five identified genetically distinct and fast-growing populations of LMB based on results from Objective 1 and 2. These fish will be transported to Piketon aquaculture facility. Eighty broodfish (sex ratio: ~1:1) will be collected from each of the populations and PIT tagged and genotyped at the OSU using microsatellite markers. Molecular genetic pedigrees will be determined and a genetic relatedness chart will be constructed for mating. Among the 80 fish, at least 30 pairs of the least-related fish will be selected for fry production. Grow-out and evaluation of the identified populations will be conducted in both pond and flow-through/recirculating tank systems after feed-training at two sites, SIUC and the OSU.

Target Audiences
Largemouth bass growers and breeders

Outreach Overview
Workshop materials will be developed to disseminate the results and train producers how to

use the genetic data and selection matrix for their own breeding programs. Results will also be published as fact-sheets and research papers. We anticipate results of Objectives 1 and 2 being made available in year 2 and objective 3 within a year of study completion.

Deliverables (Outputs)

Selection matrix and genotyping data will be compiled into a user-friendly format for largemouth bass growers and breeders, and presented through extension presentations and through the NCRAC website. Planned outputs also include publications of genetic diversity results, growth associated genotypes results, selection matrix results, and growth trials in both journal articles and extension products.

Outcomes/Impacts

- Predictions of which populations of LMB will grow the fastest to market size in the NCR have been made which have the potential to be used by largemouth bass breeders and growers in deciding what fish to acquire, breed, or purchase.
- New knowledge of genetic diversity in largemouth bass population will contribute toward the
- development of selective breeding programs.
- Discovery of genetic markers associated with largemouth bass growth will accelerate the rate of selective breeding for growth to market weight.

Impacts Summary

Relevance. — The great demand for largemouth bass (LMB) and their high selling price and growth rate (compared to other cultured species) have raised interest in their commercial culture. Slow growth has led to a call for increased efficiency of LMB growth to 1–1.5 pounds through means beyond dietary modification.

Response. — Strain evaluation and identification of the best genetically distinct largemouth bass populations was assessed by microsatellite and SNP genetic analysis for genetic diversity and growth. A complementary meta-analysis of growth data from NCR LMB population was conducted to suggested fast growing populations for broodstock development.

Results. — Predictive measures of fast growth and the identification of optimal source populations would result in an immediate impact on the economic return for many small aquaculture operations in the North Central region.

Recap. — At the completion of this project, anticipated outcomes include a description of LMB genetic diversity among commercial and public stocks available to NCR, the development of a selection markers and a matrix for fast growing LMB, and the identification of fast growing populations verified in production systems. Improved growth efficiency and shorter-time to a market size of 1.5 pounds will improve overall survival and the economics of production for NCR LMB producers.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Sunfish activities.

Technical Update

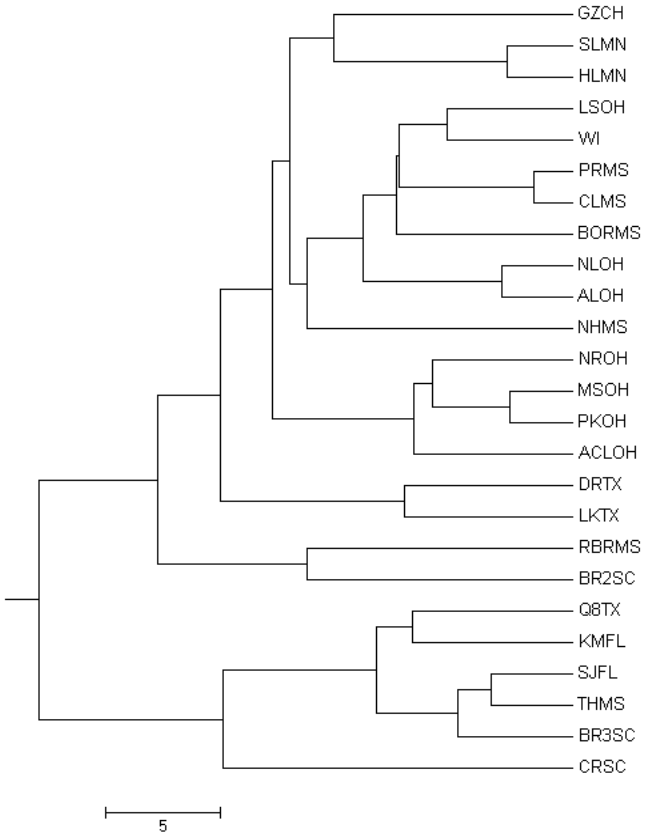

Figure 1. Phylogenetic (UPGMA) tree depicting genetic diversity of 25 LMB populations based on the allele-sharing distance. The last two letters of population abbreviations indicate the state (i.e., MN, MS, WI, OH, TX, SC, FL) or country (CH = China) from which the samples were collected.

Table 1. IGF-I allelic frequency in North Central Region largemouth bass (LMB) stocks and source hatcheries in Arkansas. A higher percentage of "A" alleles relative to "B" alleles has been correlated with higher growth in Chinese LMB populations (Li et al. 2009, 20012)[1].

Fish Farm/Hatchery	A	B	A%	B%
Little Grassy SFH-IL	7	13	**35**	**65**
Valentine SFH-NE	5	15	**25**	**75**
Fender's Fish Farm-OH	3	17	**15**	**85**
Cikana SFH-IN	3	17	**15**	**85**
Flowers Fish Farm-MO	3	17	**15**	**85**
Jake Wolf SFH-IL	3	17	**15**	**85**
J.M. Malone and Sons-AR	6	34	**15**	**85**
Veath Seven Springs-IL	2	18	**10**	**90**
TEK- Bartelso IL	2	18	**10**	**90**
Jones Fish Hatchery-OH	2	18	**10**	**90**
Southern IL. University	2	18	**10**	**90**
Cain's Fish Farm-AR	2	18	**10**	**90**
Meade SFH-KS	2	18	**10**	**90**
IF Anderson-AR	1	19	**5**	**95**
Harrison's Fish Farm-MO	1	19	**5**	**95**
Shady Lane-IL	1	19	**5**	**95**
Logan Hollow-IL	1	19	**5**	**95**
Hopper Stephens-AR	1	19	**5**	**95**
Farlington SFH-KS	0	14	**0**	**100**
Chesapeake SFH-MO	0	20	**0**	**100**
Farm Cat-AR	0	20	**0**	**100**
Arkansas Pond Stockers-AR	0	20	**0**	**100**
Fountain Bluff Fish Farm-IL	0	20	**0**	**100**
Wallace Fish Farm-KS	0	20	**0**	**100**
Black River Falls-WI	0	20	**0**	**100**

[1]Li, X. H., Bai, J. J., Ye, X., Hu, Y. C., Li, S. J., & Yu, L. Y. (2009). Polymorphisms in the 5′ flanking region of the insulin-like growth factor I gene are associated with growth traits in largemouth bass *Micropterus salmoides*. Fisheries Science, 75(2), 351-358.

Li, X., Bai, J., Hu, Y., Ye, X., Li, S., & Yu, L. (2012). Genotypes, haplotypes and diplotypes of IGF-II SNPs and their association with growth traits in largemouth bass (*Micropterus salmoides*). Molecular Biology Reports, 39(4), 4359-4365.

Project Title: Evaluate Phase II Production of Bluegill Sunfish Comparing a Least-Cost Diet Utilized in the Phase I Verification Study Compared to an "Industry Standard" for One Production Cycle [Termination Report]
Key Word(s): Nutrition/Diets
Total Funds Committed: $75,000
Initial Project Schedule: September 1, 2012 to August 31, 2013
Current Project Year: September 1, 2014 to August 31, 2015
Participants: Christopher F. Hartleb, University of Wisconsin-Stevens Point, Wisconsin; Charles E. Hicks, Lincoln University, Missouri; James Wetzel, Lincoln University, Missouri
Extension Liaison: Charles E. Hicks, Lincoln University, Missouri
Industry Liaison: Paula Moore, Jones & Eaker Farms, Missouri
Reason for Termination: Project objectives completed and funds have been terminated.

Project Objectives

1. Using consistent protocols, evaluate/determine performance of age-2 bluegill fed the diet (41% protein/<8.3% lipid) previously developed by a NCRAC funded project compared to an “industry standard” diet used in the on-going project at two distinct latitude locations in ponds for one growing season.
2. Coordinate dissemination of project results with the NCRAC Technical Committee/Extension Subcommittee. The expected deliverable will be a technical bulletin containing such detailed information as growth, production parameters, size composition, and survival using data collected over grow out to market size, i.e., the first year from the on-going plus this year’s project.

Project Summary

Growth in the North Central Region’s (NCR) aquaculture industry mirrors, and is driven by, broader U.S. and worldwide changes in the seafood industry. However domestic aquaculture production has remained about the same for the last 5 years (NMFS 2009). Aquaculture-related business in the NCR continues to be an “emerging” industry in that selection of appropriate species and associated culture practices including feed selection is evolving. As with any animal industry, feed costs can be a considerable component. Feeds often account for ≥50% of the variable costs in aquaculture budgets. To reduce variable costs there have been numerous research efforts in the NCR as well as nationally addressing the possible uses of lower-cost foodstuffs, e.g., vegetable or animal by-product as a major component of fish feeds. Clearly, substantial need exists to reduce costs and develop nutritionally adequate diets for Sunfish and other species cultured in the NCR.

Technical Summary and Analysis

Researchers from two NCR universities, Lincoln University of Missouri (LU) and the University of Wisconsin- Stevens Point (UW-Stevens Point) compared age- 2 bluegill production at densities of 7,674 sunfish/ha (2,800/acre) using two diets, the recently developed open formula versus an industry standard diet (40% crude protein and 10% lipids); both diets were produced by one common facility and distributed among the two locations. The standard diet is a commercial trout chow and the test diet is the open formula diet (Appendix 1)

developed by Robert Hayward, University of Missouri-Columbia. The Phase II least cost diet test for bluegill sunfish indicated that feed formulated with lower cost formulations can show similar results as using a currently available diet developed for salmonids which currently dominates the market.

Principal Accomplishments

Earthen ponds at LU and UW-Stevens Point (0.10-ha; 0.25-acre) were used for part or all of the study described below. Fish were stocked in (LU) ponds (6) the last week of March and feeding commenced 1 April 2012. Feeding rings were placed in each pond and fish were fed by hand twice daily except once on Saturday and no feeding on Sunday. Dissolved oxygen and water temperature were recorded daily and water quality measurements were conducted weekly.

All fish at (LU) were harvested 10/25- 26/2012. Total pond fish weights and numbers were determined and 105 fish from each pond were individually weighed and measured. Fillet weights, liver, viscera, and gills and viscera weights were determined for each fish. Thirty (30) fillet samples were taken from pond and pooled. Eighty (80) gram subsamples of fillet tissue from each pond and submitted to a Laboratory at the University of Missouri for moisture, crude protein, lipid and ash analysis. A summary of the information is included in Table 1.

Impacts

The cost differential between the two feeds would justify using the least cost diet to reduce the cost per pound of producing bluegill. The same approach to developing formulations could enable a future reduction in the reliance on marine fishery sourced feed-stuffs.

Recommended Follow-Up Activities

In year 2, there were no differences in the growth or survival of bluegill fed the standard or the least-cost diet. Therefore, by definition, the least cost diet would provide a benefit to bluegill producers by providing similar fish growth at a lower cost. The problem we encountered in year 2 was finding a feed manufacturer that would produce the least cost diet. Future follow-up activities should focus on that problem – finding a feed manufacturer that is interested and willing to produce the diet for the bluegill industry sector.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Nutrition activities.

Technical Update

Table 1. Summary of Phase II bluegill production and dressout data.

Location	Pond	TRT	Stocked Wt.	End Wt. (kg/ha)	Gain	Stocked (#/ha)	Harvest (#/ha)	% Surv.	Mn Individual Wt (0.1g)	TL mm	% On-Round	%Fillet
LU	4	IS	188.4	1263.4	65.2	7672	5502	71.7	238.5	207	89.5	23.5
LU	7	IS	189.9	1353.1	81.7	7672	5973	77.9	229.0	204	88.7	22.6
LU	12	IS	202.1	1316.2	62.1	7672	5677	74.0	241.2	207	88.9	20.6
LU	8	LC	184.6	1539.4	124.4	7672	6751	88.0	226.2	203	89.2	24.6
LU	9	LC	190.7	861.9	-17.7	7672	3650	47.6	228.2	203	89.7	21.3
LU	11	LC	171.6	1027.7	34.7	7672	4680	61.0	221.1	204	89.2	24.3
WI	PB	LC		753.7	41.3	7672	3498	45.6	214.7	201		38.6
WI	PR	IS		753. 1	39.8	7672	3314	43.2	229.5	201		35.5
WI	PF	LC		1124.8	56.0	7672	6537	85.2	171.5	225		37.4
WI	B3	LC		599. 3	45.0	7672	6475	84.4	185.3	196		36.5
WI	B4	IS		561.8	38.5	7672	5171	67.4	212.8	201		36.4
WI	B5	IS		603.8	39.3	7672	4135	53.9	208.9	200		36.7

Table 2. Summary of bluegill fillet proximate composition.

Location	Pond	TRT	Moisture	%CP	%LIPID	%ASH	LSI	VSI
LU	4	IS	76.6	20.9	1.9	1.3	1.8	8.1
LU	7	IS	78.0	20.1	1.3	1.3	1.7	8.5
LU	12	IS	76.5	21.0	1.7	1.7	1.7	8.9
LU	8	LC	77.4	20.7	1.3	1.3	1.9	7.9
LU	9	LC	77.7	20.3	1.5	1.4	1.9	7.9
LU	11	LC	76.5	20.8	2.1	1.5	1.9	8.1
WI	PB	LC	78.3	18.7	2.7	1.1		11.8
WI	PR	IS	78.1	18.3	3.1	1.1		11.9
WI	PF	LC	79.2	18.2	2.0	1.2		12.4
WI	B3	LC	77.8	18.7	3.0	1.2		11.9
WI	B4	IS	77.9	18.9	2.8	1.2		11.9
WI	B5	IS	77.3	19.3	3.1	1.2		11.8

VSI= Visceral Somatic Index LSI= Liver Somatic Index

Project Title: Assessment of Carbon Dioxide (CO_2) and Inorganic Nitrogen Compounds to Enhance Winter Kill in Natural Rearing Ponds Used for Fish Production in The North Central Region [Termination Report]
Key Word(s): Other
Total Funds Committed: $175,000
Initial Project Schedule: September 1, 2011 to August 31, 2013
Current Project Year: September 1, 2013 to August 31, 2015
Participants: Konrad Dabrowski, The Ohio State University, Ohio; Mark Gaikowski, 'USGS Upper Midwest Environmental Science Center, Wisconsin; Jason Gross, UUSGS, Northern Rocky Mountain Science Center (NOROCK), Colorado
Extension Liaison: Jeffrey Gunderson, Minnesota Sea Grant, Minnesota
Industry Liaison: Gregory Oswald, Ellendale, Minnesota
Reason for termination: Project objectives completed and funds have been terminated.

Project Objectives

1. Conduct a literature review to summarize the toxic effects of carbon dioxide (CO2) and inorganic nitrogen compounds (e.g. N2, NO2−, NH3, etc.) on fish with an emphasis on common carp *Cyprinus carpio*, black bullhead *Ameiurus melas* and walleye *Sander vitreum*.
2. Estimate the cost per acre of pond treatment using either CO2 or inorganic nitrogen compounds to enhance winter kill conditions during late winter periods in the North Central Region (NCR).
3. Consult with EPA to determine the registration eligibility and requirements for the use of CO2 or inorganic nitrogen compounds to enhance winter kill conditions.
4. Determine, through laboratory study, application rates required of CO2 or inorganic nitrogen compounds to enhance winter kill conditions to remove unwanted fish from natural rearing ponds. Studies required for the registration of CO2 or inorganic nitrogen compounds to enhance winter kill conditions will be conducted according to GLP regulations (40CFR160).
5. Evaluate, through laboratory pond experiments, the efficacy of laboratory-derived application rate data for CO2 or inorganic nitrogen compounds to enhance winter kill conditions.
6. Collect late winter water chemistry condition data in representative NCR natural rearing ponds.
7. Obtain an experimental use permit (EUP) from the EPA and appropriate state regulatory agencies to conduct experimental applications of CO2 or inorganic nitrogen compounds, singularly or in combination, to enhance winter kill conditions in natural rearing ponds to remove populations of unwanted fish.
8. Compile data into final study reports suitable for submission to the EPA to support potential approval of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.
9. Summarize results into appropriate extension materials for dissemination to NCR aquaculturists.

Project Summary

Fish culturists are often left with a pond containing a few undesirable fish at the end of a year. These undesirable fish can lead to decreased yields and profits during subsequent years. Current strategies to control these undesirable fish require culturists to use expensive chemicals and to properly remove carcasses. These studies determined thresholds of the combined effects of readily available CO_2/inorganic nitrogen compounds on survival of fishes to enhancing or manage winterkill conditions for fish culturists. It required 0.37 mg/L ammonium chloride when oxygen levels were very low to achieve total mortality in some fishes, or required 495 mg/L CO_2 to achieve total mortality. The use of diffused gases and/or inorganic nitrogen compounds appear to offer alternatives to commonly used toxicants for aquaculture operations where undesirable fish species need to be controlled. The use of CO_2 would cost approximately $244 and ammonia chloride would cost $264 to treat an acre foot of water, while rotenone would cost $140 to treat that same volume. However, since fish exposed to rotenone are classified as hazardous, there is an additional cost of removal of the carcasses following a rotenone treatment. Using either inorganic nitrogen or CO_2 may be a viable alternative to the use of chemicals for fish control. Currently, the registration of CO_2 as a fish control is underway.

Technical Summary and Analysis

Objective 1. — A manuscript is in the advance stage of development. The first draft was prepared as introduction section to the dissertation by T. Parker which was defended in August 2013. The manuscript focuses on toxicity of ammonia nitrogen compounds at different oxygen levels in common carp and discusses, among other aspects, the changes to the gill tissues associated with the ammonia/hypoxia exposure. Data obtained for common carp is being finalized into a separate manuscript prepared for submission to “Aquatic Toxicology”. Toxicity of ammonia to carp, percids, salmonids, and catfish will be compiled and presented with a literature review to make a white paper to be reported to NCRAC.

The literature review for the use of CO_2 as a control agent for undesired fish species is currently under review by U.S. Geological Survey and U.S. Fish and Wildlife Service partners for submission for publication to Fisheries. This literature review incorporates findings from preliminary experimental work with multiple fish species and gives a comprehensive description of CO_2, its use and efficacy.

Objective 2. — The cost per acre foot for pond treatment was calculated the amount of CO_2 to produce mortality observed in pond trials (Objective 5). The cost of CO_2 gas was $0.34/kg ($0.16/lb) while liquid CO_2 was $0.23/kg ($0.11/lb). Therefore, the costs to treat an acre foot of pond with CO_2 gas was $361.35 and $244.16 if treated with liquid CO_2. The actual cost for fish producers will be dependent upon local distributors.

The cost per acre foot for pond treatment was calculated for the amount of ammonia chloride to produce mortality observed in pond trials (Objective 5). The cost of ammonia chloride was $1.36/kg ($0.62/lb). The cost to treat an acre foot of pond with this ammonia chloride was $105.95; however, 100% mortality was not achieved. A 2.5 times of the dose used in our trials (i.e. adding 62.5 kg [28.41 lb] rather than 25 kg [11.37 lb]) would be expected to produce 100%

mortality. This would increase the costs to $264.88 for treating an acre foot of pond with ammonia chloride to produce 100% mortality. However, the actual cost will be dependent upon local distributors.

For comparison, the cost per acre foot for pond treatment was calculated for the amount of rotenone to produce 100% mortality. This piscicide would cost approximately $140.00 to treat an acre foot of pond. However, the cost of removal and disposal of rotenone-exposed fish are not included. These additional costs could substantially increase the total costs for use of this chemical and would be minimized in using CO_2 or ammonia chloride. Thus, both CO_2 and ammonia chloride may be viable alternatives to the use of rotenone for the removal of fish remaining in ponds during late winter.

Objective 3. — Completed.

Objective 4. —
CO2 - Studies have been completed. A significant interaction between treatment and species was observed. The interaction was a result of differences in mortality between species at specific treatment levels. In the common carp (CAP) and channel catfish (CCF) trials, mortality in both the 380 ppm CO2 and 495 ppm CO2treatment groups was significantly higher than in the control, 75, 150, 225, and 300 ppm CO2 groups, but mortality in the 495 ppm CO2treatment was higher than in the 380 ppm CO2 treatment. There was no difference in mortality between the 225 ppm CO2 and 300 ppm CO2 treatment groups in the CCF trials. There were no differences in mortality in any treatment groups between CAP and CCF.
Inorganic

N - Studies have been completed. There was significant interaction between ammonia concentration and dissolved oxygen is significant in yellow perch which indicates that ammonia toxicity is increased by reduced oxygen content in the water for yellow perch. Yellow perch mortality for fish exposed to 0.37 ± 0.01 ppm NH3-N was 6.25 ± 6.25%, 93.75 ± %, and 100.0 ± 0.0% in normoxia, moderate-hypoxia, and severe hypoxia, respectively. When exposed to 0.23 ± 0.03 ppm NH3-N mortality for the three oxygen treatments, in the same order, were 2.50 ± 2.50%, 22.5 ± 13.1%, and 72.5 ± 24.3%. All rainbow trout survived during exposure to 0.15 ± 0.02 ppm NH3-N. However, during exposure to 0.26 ± 0.02 ppm NH3-N, rainbow trout experience significantly higher mortality in severe hypoxia. Rainbow trout experienced 6.25 ± 8.84%, 7.81 ± 7.86%, and 48.4 ± 25.7% in normoxia, moderate-hypoxia, and severe hypoxia, respectively.

Objective 5. — Studies have been completed. A controlled outdoor pond study to assess the use of CO_2 and ammonia chloride for enhancement of winter kill conditions was performed at the U.S. Geological Survey Upper Midwest Environmental Sciences in La Crosse, Wisconsin. In early October of 2012, eight fish-free 0.04-ha (0.1-acre) outdoor rectangular ponds were filled with well water to a mean depth (± SD) of 96 ± 4 cm (37.8 ± 1.57 in). On October 11, 2012, each pond was stocked with 100 common carp (5.0 - 7.5 cm [2.0 - 3.0 in]) and 100 channel catfish (7.5 - 20.3 cm [3.0 - 8.0 in]). Ponds were left to freeze under natural conditions.

On February 19, 2013, a 12.7 cm (5 in) diameter hole was drilled through the ice in the center of each pond. Temperature and dissolved oxygen were measured at the surface and bottom of each pond and pH was measured at the bottom of each pond. Carbon dioxide was measured with a commercially available titrant kit.

On March 7, 2013, one 12.7 cm (5 in) hole was drilled in the northeast corner and a similar size second hole in the northwest corner of each pond. One hole was used to pump water (approximately 0.264 L/min [30 gal/min]) from the bottom of the pond, treated with the chemical and then returned through the second hole just below the ice. Each of three randomly selected ponds was injected with ammonia chloride (Prince Agri Products, Inc., Quincy, IL). At the side of the pond, 55.12 lbs (25 kg) ammonia chloride was dissolved in approximately 208 L (55 gal) of pond water and injected into each of the at approximately 113 L/m flow (29.85 gal/min). This amount of ammonia chloride was used to achieve approximately 85 ppm ammonia, which is more than double the 31.7 ppm required to reach 0.85 ppm NH_3-N at a pH of approximately 9.0 at 4°C (39.2°F; Piper, 2010).

Between March 8 and March 10, 2013, three other randomly selected ponds were injected with 48.4 ± 2.9 kg (106.7 ± 6.39 lbs) of CO_2 delivered as a gas at a rate of 25 L (6.60 gal) at approximately 113 L/min flow (29.85 gal/min). This amount of amount of $CO_2$was used to achieve a nominal concentration of 150 ppm CO_2, which was expected to overcome alkalinity of the pond water and result in toxic levels of free CO_2, greater than 25 ppm.

On March 11, 2013, a 12.7 cm (0.12 ft) diameter holes were drilled through the ice in the center of the pond, one on the north end and one on the south end of each pond. Temperature and dissolved oxygen were measured at the surface and bottom of each pond and pH was measured for each pond. Dissolved CO_2 was measured with a commercially available titrant kit (CHEMetrics, Inc., Midland, VA). Ponds were then left to thaw under ambient conditions. On April 11, 2013, all ponds were free of ice and immediately drained. All live fish were harvested from each pond and counted.

All water quality parameters (i.e. pH, temperature, dissolved oxygen, ammonia and CO_2) and the number of surviving carp and catfish are reported as means with standard deviations. Water quality parameters were compared between treatments pre- and post-treatment using ANOVA. Initial, pre-treatment, water qualities were similar among ponds. Water temperatures were 3.11 ± 0.67°C (37.60 ± 1.38°F). All ponds were slightly basic, 9.14 ± 0.34. Ponds treated with CO_2 had the greatest pH, 9.57 ± 0.19 and differed from that in ponds treated with ammonia (8.83 ± 0.08) or left untreated (8.96 ± 0.08). Dissolved oxygen was found to be super saturated in all ponds, 219.5 ± 35.9%. No dissolved CO_2 or ammonia was detected in any of the ponds prior to treatment.

Differences were observed in water quality parameters post-treatment. Water temperatures were lower in all ponds when measured following treatments on March 11, 2013 then when measured during pre-treatment. Water temperatures were lowest in ponds treated with CO_2 (0.47 ± 0.12°C [32.85 ± 0.21°F]), but similar between control ponds and ponds treated with ammonia 1.58 ± 0.21°C (34.84 ± 0.38°F) and 1.43 ± 0.23°C (34.57 ± 0.42°F) respectively. pH did not change in either the control ponds or ponds treated with ammonia. The pH in ponds treated with CO_2

dropped from 9.57 ± 0.19 to 6.13 ± 0.11, thus the CO_2-treated ponds had lower pH than either the control ponds or ponds treated with ammonia. DO did not change in control ponds or ponds treated with CO_2, but increased to 32.94 ± 3.13 ppm in ponds treated with ammonia. No differences in DO were found between any of the treatments on March 11, 2013. Total ammonia was greatest in ponds treated with ammonia chloride, 12.60 ± 11.18 ppm, but was only a fraction of the 31.7 ppm needed to produce 100% mortality. Total ammonia levels in ponds treated with CO_2 or untreated had very low levels of total ammonia, 0.22 ± 0.17 ppm and 0.13 ± 0.12 ppm respectively. Dissolved CO_2 was detected in only those ponds treated with CO_2.

The number of fish surviving varied among treatments. No live common carp were found in one of the control ponds, while 90 channel catfish were alive in that same pond. Thirty-five common carp and 9 channel catfish were alive in the other control pond. Survival ranged from 0 to 35 for common carp and from 1 to 36 for channel catfish in ponds treated with ammonia. Only a single common carp and no channel catfish survived in all ponds treated with CO_2. Ponds treated with CO_2 did have greater total fish mortality than the control ponds. Ammonia chloride had no effect on mortality at levels used in our study.

Objective 6. — Completed - Long term ice cover was never present in central Ohio and therefore winterkill conditions were not observed.

Objective 7. — Completed - UMESC held an initial coordination meeting with EPA to discuss whether the use of CO_2 will require EUP to allow its use to enhance winterkill conditions. Depending on the size and scope of the application, it is likely that EUP would be required prior to registration. As part of its efforts to assess the use of CO_2 in barriers to deny aquatic invasive species access to critical habitat, UMESC will be evaluating the required registration information required to register CO_2 for use to control aquatic nuisance species.

Objective 8. — Completed - UMESC is coordinating with EPA to determine the information required to register CO2 for uses to control aquatic nuisance species. UMESC and its collaborators on this project will compile data reports of project generated information to meet those registration requirements, where appropriate.

Objective 9. — Completed - Extension materials will be prepared pending coordination with EPA to determine registration requirements. Extension materials cannot be prepared and disseminated until a determination is made regarding the registration requirements of CO_2 or inorganic nitrogen compounds.

Principal Accomplishments

A simple method for delivering CO_2 or ammonia below ice was developed. The greatest return on investment for this project will be in the removal of undesirable fish from both public and private aquaculture ponds. Removal of remaining fish will enhance production in those ponds. Results of the experiments, where appropriate, will be presented at scientific meetings and extension workshops and may be published in scientific journals, extension bulletins, or NCRAC fact sheets and bulletins. Research results will also be disseminated

through the NCRAC Annual Progress Reports. These reports are available on the NCRAC Web site http://www.ncrac.org .

Impacts:

- The approval of CO_2 and/or ammonia for the removal of unwanted fish in natural rearing ponds will be a significant benefit to the public and private aquaculture producers. Removal of these fish will enhance production in these ponds.
- Approval of CO_2 and/or ammonia as a chemical for the control of unwanted fish species is contingent on providing evidence on the effectiveness of these chemicals to produce mortality in targeted fishes. Thus, an initial study required includes the establishment of efficacy of both CO_2 and ammonia. Trials have been completed.
- Both CO_2 and ammonia produced mortality in laboratory and field trials. Cost of both of these chemicals is greater than that required to treat ponds with the registered piscicide rotenone. However, the use of CO_2 or ammonia as a fish toxicant will not produce dead fish that will require special disposal like those killed with rotenone. The removal of these unwanted fish is critical to improvement of efficiency of public and private fish producers.
- CO_2 infusion under ice was effective in eliminating channel catfish and common carp during under ice exposures.

Recommended follow-up Activities: Future studies should focus on the development of an injection system for deliver CO_2 under ice. Studies should also look the relationship between dose time.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Other activities

Project Title: Snail Management/Grub Control* [Termination Report]
Key Word(s): Other
Total Funds Committed: $20,500
Initial Project Schedule: September 1, 2007 to August 31, 2009
Current Project Year: September 1, 2013 to August 21, 2014
Participants: Richard D. Clayton, Iowa State University, Iowa; Christopher F. Hartleb, University of Wisconsin-Stevens Point, Wisconsin; Todd Huspeni, University of Wisconsin-Stevens Point, Wisconsin; Joseph E. Morris, Iowa State University, Iowa State University; Gregory W. Whitledge, Southern Illinois University-Carbondale, Illinois
Extension Liaison: Joseph E. Morris, Iowa State University, Iowa
Industry Liaison: Rex Ostrum, Nebraska

Project Objective

2. Assemble an updatable snail management guide which includes a literature review of known control options, a method of determining snail infestation levels in any water system, and a set of standard operating procedures to reduce snail populations and trematode infestations based on the research cited in Objective 1.

Project Summary

With the long history of trying to control snails in fish ponds and types of control mechanism being used (mechanical/chemical/predatory), a literature review was undertaken to compile the information to date. This list of references is from journal articles only. The references are sub-divided by the type of control that the researcher's evaluated or commented on: mechanical, chemical, or biological control. This information was then combined with information garnered from this project to produce a detailed presentation that is now available to North Central Region (NCR) aquaculture community.

Technical Summary and Analysis

Objective 2. — A search has been completed by Iowa State University staff to review literature to date concerning the three main control methods for snails: biological, chemical, and mechanical. This information was combined with information garnered from this research project to develop detailed on-line presentation for fish producers to access and obtain information potentially relevant to their snail problems. Among the various options, information regarding effectiveness, legal implications, and potential for impact on pond general ecology, e.g., zooplankton dynamics in fish fingerling ponds, will be listed. The detailed presentation 'Snail Management in Culture Ponds' is hosted on the revised North Central Regional Aquaculture Center (NCRAC) Web site – Aquatic Biological Management http://www.ncrac.org/files/presentation/file/Snail%20Management%20in%20Culture%20Ponds.pdf The literature review 'Review of Snail Control in Fish Ponds' http://www.ncrac.org/files/biblio/snail_control_litrev.pdf is also available on the same web site.

* This Progress Report is for the second objective of this project. A Project Component Termination Report for the first objective is contained in the 2009-10 Annual Progress Report. This is a project that had two years of funding and is chaired by Gregory W. Whitledge. It began September 1, 2007.

Additional information on grubs in freshwater fish is available in the NCRAC Technical Bulletin #115 'Biology, Prevention, and Effects of Common Grubs (Digenetic trematodes) in Freshwater Fish', http://www.ncrac.org/files/biblio/TB115.pdf . Since the initiation of this project, an updated factsheet on snails and associated grubs has been developed at Texas A&M University and is available at http://fisheries.tamu.edu/files/2013/09/My- fish-have-grubs-final.pdf .

Principal Accomplishments

Results from this project have been used to provide an updated review of snail control methods available to the aquaculture community. Although to date there is no one ideal management solution to controlling the snail population in aquaculture ponds, aquaculturists should consider the following:

- Prevention of snail infestation when possible.
    - Use of approved aquatic herbicides or grass carp (consult state-specific regulations to decrease the amount of submerged vegetation including filamentous algae.
    - Drying pond bottoms between crops.
    - Use of flow in side tanks to limit infestations.
- Use care in the use of chemical controls as many will affect the cultured fish directly.
    - Chemical treatments often effective along pond margins but small fish may be susceptible.
    - Use of lime to modify water pH can affect both adult fish and their offspring through direct and indirect effects as well as low oxygen levels associated with decaying vegetation.
    - Applications copper sulfate (combined with citric acid) are effective control measures but can direct or indirect effects on cultured fishes; previous research notes the effect of 0.25 mg/L of copper sulfate can kill desired zooplankton prey for larval fishes.
    - Prior to chemical control use, culturists need to check total alkalinity of the culture ponds to help decrease deleterious effects from lime or copper sulfate applications.
- Consider use of biological controls for long-term controls.
    - Redear Sunfish are effective in controlling Physa but not rams-horn snails until they are fully matured.
    - Use of hybrid Redear Sunfish can help to reduce snail populations over the culture period.
    - Other possible predators include crawfish, blue catfish, freshwater drum and freshwater prawns.

Recommended Follow-Up Activities

A yearly review of literature pertaining to grub and snail management should be undertaken

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output
for all NCRAC-Funded Other activities.

Project Title: Developing Genetically Fast-Growing Monosex Populations in Bluegill [Progress Report]
Key Word(s): Sunfish
Total Funds Committed: $160,000
Initial Project Schedule: September 1, 2013 to August 31, 2015
Current Project Year: September 1, 2014 to August 31, 2015
Participants: Charles E. Hicks, Lincoln University, Missouri; Han-Ping Wang, The Ohio State University, Ohio; James Wetzell II, Lincoln University, Missouri
Extension Liaison: Charles E. Hicks, Lincoln University
Industry Liaison: Curtis Harrison, Harrison Fisheries, Inc., Missouri

Project Objectives

1. Identify additional super - males and performance - selected females from existing populations.
2. Create all - male bluegill populations by crossing super - males with females of selected and non - selected stocks.
3. Rear populations at two or more locations in the NCR.
4. Compare sex ratios and production characteristics of sub - populations as based on maternal stocks.

Deliverables

1. Characterize the performance characteristics and sex ratios of super - male/performance - selected cross.
2. Characterize the economic cost benefits of culturing the super - male/performance - selected cross.
3. Publication of results in journal article, and extension publications (i.e., factsheets, research tours).

Project Summary

Improving the growth rate and broodstock of bluegill and its hybrids has been ranked as one of the top priorities in USDA-NCRAC. The proposed research will specifically address the needs identified by that agency. The results of this research can be expected to advance our understanding of sex-determining mechanisms in fish. Further, using this information, we expect to be able to obtain super male broodfish. By the completion of the proposed research, we expect to generate genetically fast- growing all-male populations by crossing super males with genetically improved females. Not only will a monosex culture be expected to produce the greatest biomass in a given period of time, but also all male bluegill culture may promote growth by reducing the metabolic cost of sexual growth and reproduction. This will benefit fish farmers by increasing the efficiency and profitability of sunfish aquaculture production in the U.S.

Anticipated Benefits
Improving the growth rate and broodstock of bluegill and its hybrids has been ranked as one of the top priorities in USDA-NCRAC. The proposed research will specifically address the needs identified by that agency. By the completion of the proposed research, we expect to generate genetically fast- growing all-male populations by crossing super- males with genetically improved females. These outcomes will enable us to develop GMB-producing broodstock and mass production of monosex populations. Not only will a monosex culture be expected to produce the greatest biomass in a given period of time, but also an all- male bluegill culture may promote growth by reducing the metabolic cost of sexual growth and reproduction. Therefore, this will benefit fish farmers by increasing the efficiency and profitability of sunfish aquaculture production in the U.S. The impact of this project will be primarily via the delivery of fast-growing all-male bluegill population to fish farmers in Ohio, the Midwest, and other states. The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs. A successful creation of genetically male bluegill strains would have a tremendous impact on the sunfish aquaculture industry by increasing growth rate of 30-35% (Wang and Hayward 2006) and saving energy expenditure of 20-30% for sex growth.

Project Progress
Objective 1. — Progeny test for all-male populations using improved fish from Lincoln University of Missouri was continued. Temperature effects on sex ratio have been found in some geographic populations: producing more males in high temperature, more females in low temperature. The findings were published in Aquaculture. Follow-up investigation using four different geographic populations strongly suggests that both temperature-dependent sex determination (TSD) and genetic sex determination (GSD) exist in bluegill. This paper is in revising phase being considered by The Biological Bulletin for publication.

Objective 2 - Three batches of improved fish have been transported OSU aquaculture facility from Lincoln University. Twenty-four selected and improved females and 24 selected male were stocked into twenty-four 400 L round tanks with flow-through well water at OSU South Center Wet Lab. Water temperature and photoperiod were set at 25°C and 16 h light: 8 h dark, and an artificial spawning nest was placed in each tank. Fish were checked twice daily. Nests with eggs were placed in the bottom of aerated 400-L tanks with flow-through well water for incubation. Fifteen pairs spawned and 12 batches of expected all-males were produced. Once the sex is confirmed, the fish will be distributed to two or locations and compare sex ratios and production characteristics.

Target Audiences
Aquaculture Farmers.

Outreach Overview
Nothing to report yet

Deliverables (Outputs)
Nothing to report yet

Outcomes/Impacts
The impact of this project will be primarily via the delivery of fast-growing all-male bluegill populations to fish farmers in Ohio, the Midwest, and other states. The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs. A successful creation of genetically male bluegill strains would have a tremendous impact on the sunfish aquaculture industry by increasing growth rate of 30-35% and saving energy expenditure of 20-30% for sex growth.

Impacts Summary
Relevance. —Despite this opportunity, rapid expansion of the bluegill aquaculture industry has not occurred in this country. One reason in particular hindering expansion has been the relatively slow growth of currently cultured populations of this species.
Response. — Monosex culture would hold considerable potential as a method to increase the efficiency and profitability of bluegill food aquaculture by improving growth rate, and eliminating the problem of prolific reproduction, precocious maturity and their consequences. We have started a project to create all-male bluegill populations by crossing super-males with females of selected and non- selected stocks.
Results. — A successful creation of genetically male bluegill strains would have a tremendous impact on the sunfish aquaculture industry by increasing growth rate of 30-35% and saving energy expenditure of 20-30% for sex growth.
Recap. — The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs.

Publications, Manuscripts, Workshops, and Conferences
See the Appendix for a cumulative output for all NCRAC-Funded Sunfish activities.

Project Title: Determination of Production Parameters of Selected Yellow Perch Lines at Commercial Densities in Ponds at Two or More Facilities in the North Central Region [Termination Report]
Key Word(s): Yellow Perch
Total Funds Committed: $150,000
Initial Project Schedule: September 1, 2010 to August 31, 2013
Current Project Year: September 1, 2014 to August 31, 2015
Participants: Christopher F. Hartleb, University of Wisconsin – Stevens Point, Wisconsin; Laura G. Tiu, The Ohio State University, Ohio; Geoffrey K. Wallat, The Ohio State University, Ohio; Han-Ping Wang, The Ohio State University, Ohio
Extension Liaison: Geoffrey K. Wallat, Ohio State University, Ohio
Industry Liaison: Charles E. Hicks, New Bloomfield, Missouri
Reason for Termination: Project objectives completed and funds have been terminated.

Project Objectives

1. Using consistent protocols, assess survival and growth rate of two replications of first
   - year fingerlings of improved lines of yellow perch as compared to fingerlings from local brood stock (feed - trained fingerlings to be stocked at 60,000/acre (150,000 fish/ha).
2. Using consistent protocols, assess 2nd year survival, growth rate, and market parameters (production, fillet yields, percent market size) of both replications of improved lines of yellow perch as compared to local fish.
3. Disseminate results to industry and to end - user customers via fact sheets, scientific publications, and an on - farm field day.

Project Summary

Yellow perch has its unique niche market in the Great Lake Region and the North Central Region (NCR). Despite this opportunity, rapid expansion of the yellow perch aquaculture industry has not occurred in these regions. One reason in particular hindering expansion has been relatively slow growth of currently cultured populations of this species. Using current yellow perch strains, only 60% of the fish cultured in aquaculture operations reach market size in a normal growth cycle (16 months), with the rest being below market size. This is an inefficient use of resources, feed, and operational costs, and leads to marginal profits at best. Therefore, improving and promoting yellow perch growth and aquaculture using new technology will significantly improve the profitability of fish farmers. Genetic improvement of aquaculture species offers a substantial opportunity for increasing production efficiency, health, production quality and, ultimately, profitability in aquaculture industries. The Ohio State University has developed genetically improved yellow perch. On-station and on-farm tests are important steps for commercialization of genetically improved strains.

Technical Summary and Analysis

Objective 1.—

Ohio: Researchers conducted replicated tests of the selected line of fish vs. the local-strain using two types of rearing tests: 1) at the Piketon Station (selected line and a local-strain were reared in

separate ponds, each having two replicates) and at Mill Creek (MC) Perch Farm (selected line and a local-strain were raised communally). Eight molecular markers were used to assign selected and local-strain yellow perch to their family of origin for communal rearing.

In the first year the selected line of fish grew significantly larger than local perch native to the farm in two communal ponds at MC Perch Farm. The selected line fish outweighed the local strain by 32.00% on average at the end of the Year 1 test. Fingerling survival in the MC Perch Farm's communal ponds with improved fish was as high as they have ever experienced. In the Piketon ponds, the selected line fish exhibited a 27.16% higher survival rate and a 22.01% higher production than the local Ohio strain by the end of October of Year 1. Although the 27.16% higher survival rate of the selected line fish resulted in a significantly higher density and lower feed rations, these fish still had a higher mean body weight than the local Ohio strain.

Wisconsin: Approximately 15,000 feed-trained selected line fingerlings were hauled from OSU Piketon Research Facility to the WI NADF with <1% mortality. All ponds were harvested in October of year 1. The selected line fish from two ponds averaged 125.1mm (4.93 in) and 25.6 g (0.90 oz) and 120.3 mm (4.74 in) and 22.5 g (0.79 oz), respectively. The WI-strain perch from two ponds averaged 118.6 mm (4.67 in) and 20.1 g (0.71 oz), and 111.8 mm (4.65 in) and 17.9 g (0.63 oz), respectively. Average fingerling survival was 92.0% for selected line and 72.0% for WI local strain. Although the 20.0% higher survival rate of the selected line fish resulted in a significantly higher density and lower feed rations (rations were calculated based on the same assumed survival rate for all the ponds), they still grew 26.60% faster than the unimproved fish.

Objective 2.—
Ohio: For MC Perch Farms, out of 240 fish that were family-origin identified from each of the two ponds, selected line fish accounted for 71.25% and 51.25% in each of the individual ponds with an average of 61.25% which suggests that selected line fish and MC perch had survival rates of 61.25% and 38.75%, respectively. The local Ohio strain at MC Perch farms from two ponds averaged 204.0 mm (8.03 in) and 105.6g (3.72 oz), and 193.9 mm (7.63 in)and 92.9 g (3.28 oz), respectively. The selective line fish from two ponds averaged 218.1 mm (8.59 in) and 142.7 g (5.03 oz), and 212.0 mm (8.35 in) and 129.9 g (4.58 oz), respectively. The selected line fish outweighed the local strain by 35.16% and 39.90%, respectively, with an average of 37.53%, at the end of the Year 2 test in two communal ponds.

In the Piketon ponds, selected line fish exhibited a 12.30% higher survival rate and a 42.07% higher production than the local Ohio strain by the end of October of Year 2. Although the 12.30% higher survival rate of the improved fish resulted in a significantly higher density and lower feed, the improved line still grew 25.50% faster than the unimproved fish. There was no significant difference in dress-out percentage between improved line and local Ohio strain.

Wisconsin: All four ponds were harvested in September, 2013. A total of 29 and 40 selected line perch were harvested from two ponds, and 40 and 21 WI local strain fish were harvested from two ponds. The selected line perch averaged 198.17 mm (7.80 in) and 111.90 g (3.95 oz) and

202.0 mm (7.95 in) and 114.28 g (4.03 oz), respectively. WI strain perch from two ponds averaged 189.53 mm (7.46 in) and 89.85 g (3.16 oz) and 197.24 mm (7.77 in) and 100.91 g (3.56 oz), respectively. Length and weight were both significantly ($p<0.001$) greater for selected line yellow perch compared to WI local strain yellow perch. Fillet weight was significantly ($p<0.001$) greater for selected line perch, while percent fillet yield was significantly greater for WI local strain yellow perch.

Northern Wisconsin experienced severe and prolonged winter weather in 2013 that resulted in pond ice cover that did not dissipate until early May. This extreme weather conditions in turn was like the significant cause in the poor survival of both groups of fish. Due to the poor survival of all fish, total production and percent market size were unable to be calculated.

Objective 3.— Farm test progress and results have been disseminated to industry and to end-user customers via three articles published in newsletters, journals and website.

Principle Accomplishments

The Ohio State University has established selective breeding program for genetic improvement of growth in yellow perch. The third generation of genetically improved perch has been created. Farm tests of the genetically improved lines were conducted in three sites in NCR. Farm tests showed the improved fish had ~35% faster growth rate and ~20% higher survival rate than unimproved fish. Fish farmers could use the genetically improved perch to increase production efficiency of yellow perch aquaculture. Also, marker aided cohort selection has been developed and tested. This effective breeding method can be used in other species.

Impacts

Genetically improved yellow perch that grow ~35% faster than unimproved fish would have a tremendous positive impact on the NCR yellow perch aquaculture industry. The impact of this project will be primarily through the delivery of the superior yellow perch strains to farmers for use in a wide range of culture and exposure conditions across the NCR. Fish farmers could use the genetically improved perch to significantly improve production efficiency of yellow perch aquaculture. The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs by using genetically improved strains.

Recommended Follow-Up Activities

All female population of yellow perch should be developed using currently improved lines to further improve growth. Cost recovery basis for these improved lines of stock should be developed through either institutional or commercial outlets.

Project Title: Develop Systems and Diet Strategies to Reduce Yellow Perch Larval Mortality Burst in Indoor Recirculating Aquaculture Systems [Progress Report]
Key Word(s): Yellow Perch
Total Funds Committed: $190,000
Initial Project Schedule: September 1, 2013 to August 31, 2015
Current Project Year: September 1, 2014 to August 31, 2015
Participants: Gregory Fisher, University of Wisconsin-Stevens Point, Wisconsin; Christopher F. Hartleb, University of Wisconsin-Stevens Point, Wisconsin; D. Allen Pattillo, Iowa State University, Iowa; Han-ping Wang, The Ohio State University, Ohio
Extension Liaison: Allen Pattillo, Iowa State University
Industry Liaison: Rich Lackaff, V - Bar Aquaculture, NE

Project Objectives

1. Develop system(s) to address physical and behavioral barriers to enhance mass production and survival of yellow perch (YP) from onset of first feeding up to 70 days.
2. Develop strategies to increase survival of fry and larvae of yellow perch reared indoors using different feeding regimens.

Deliverables

1. Develop modules for self/group training for YP aquaculturists. Modules should be prepared at the initiation of the project and updated to include new procedures/protocols learned from the project.
2. Prepare an overall report of the findings including an executive summary.

Project Summary

In culture conditions, there are several critical factors affecting survival of larval yellow perch, including small mouth gape, dependence on live food organisms, non-feeding behavior, non-inflation of the gas bladder, clinging behavior, and cannibalism. Despite the availability of high quality feeds for small larvae, mainly formulated for marine species, the acceptance, growth and survival of larval yellow perch fed formulated diets as starting food are still highly variable and rather unsatisfactory. This project is investigating the development of systems and strategies to enhance mass production and survival of yellow perch from onset of first feeding up to 70 d, the critical period for yellow perch in recirculating aquaculture systems.

Anticipated Benefits

Results garnered from this commercial- scale research will be incorporated into an overall report, including executive summary, about culture strategies and protocols that can be used to increase the survival or larval (fry) yellow perch in indoor recirculating systems using culture methods and feeding regimens that maximize mass production. These new methodologies will greatly improve larval yellow perch survival and help feed the growing RAS production of yellow perch food fish, an important aquaculture species in the NCR.

Project Progress

Objective 1. (UWSP-NADF). UWSP: Non-feeding behavior, non- inflation of the gas bladder, clinging behavior, and cannibalism were examined using a series of methodical experiments

evaluating the effects of: 1) Turbid water – three treatments of clear (0 NTU), slightly (50 NTU), and turbid (100 NTU) water; 2) Water surface spray – three treatments of no flow (0 L/min, 0 gal/min), moderate (0.4 L/min, 0.11 gal/min), and high (0.8 L/min, 0.21 gal/min) water flow; 3) Tank color – three treatments of white, blue, and black interior colored tanks. No differences ($p>0.05$) in growth, length and weight, were observed every two weeks nor were differences observed after 70 days for larval yellow perch raised in different color tanks (blue, white and black). Significant differences ($p<0.05$) in growth, length and weight, were observed for larval yellow perch raised under different water surface spray. High spray velocity (0.8 L/min; 0.21 gal/min) resulted in the greatest growth, both length and weight, especially when comparing no flow, poorest length gain, to high flow. No flow and moderate flow (0.4 L/min; 0.11 gal/min) showed similar weight gain with high flow having the greatest weight gain. Significant differences ($p<0.05$) in growth, length and weight, were observed for larval yellow perch raised in different turbidity levels. Slightly turbid water (50 NTU) resulted in the greatest growth, both length and weight, when compared with clear water (0 NTU) and turbid water (100 NTU). No differences in growth of larval yellow perch were seen between clear water and turbid water conditions.

Objective 2 (OSU). Objective 2.1 - Other than improving commercial-scale rotifer and *Artemia* production system established last year, the 24/7 auto-feeders were also optimized. Live feed production systems were improved either through reducing feeding times for rotifer or reducing production cost, e.g. decreasing salinity for *Artemia* hatching from 25-30% to 12-15%, without any compensation of output. The regular air stones in the stocking buckets of live feeds (rotifer/*Artemia*) in the auto-feeders were replaced by round, flat air stones because we found a considerable proportion of live feed sink on the bottom and were not pumped out to fry tanks last year. This improvement will allow more than 90% of live feed to be delivered to fry tanks. Objective 2.2 - Seven feeding regimes were tested in 2015, with each having two replicates, via combination of mouth-opening prey (rotifer or *Artemia*), initial age of weaning, duration of weaning, duration of co-feeding, and different larvae formula feed (Otohime, B1 200-360 µm and B2 360-600 µm; Zeigler 150-250 µm and 250-400 µm). Several related studies was completed: 1) by monitoring egg size produced by different strains/families, we have identified some strains/families that produced significantly larger-mouth gape progeny and larger eggs than others; 2) variation of egg size is dramatically different among strains of our genetically improved fish, indicating there is a large range of selection for large eggs; 3) we found predation and ingestion of prey at the beginning of feeding is limited by the mouth gape in fish larvae which determine larvae survival.

Examination of food in digestive tract, combining with mouth size as well as total length data proved that the yellow perch larvae were able to ingest live feed, either rotifer or *Artemia*, when only total length reached 6 mm and mouth gape reached 0.6 mm, regardless the age of larvae, which was usual from 1 to 7 DPH. We also found that many rotifer and *Artermia* can survival (moving) in the digestive tract (including stomach and intestinal tract) when the larvae were younger than 17 DPH (total length less than 20 mm), indicating that enzymes the digestive track had not developed well as older fry.

Swim bladders started to inflate as early as 4 DPH, and were correlated with the size of fish. When pooling all families together, we found that the swim bladder would inflate when larvae the size reached 6.7 - 9.7 mm, regardless the age of fish. Yolk disappeared between 7 and 11 DPH for most of larvae. For one family, yolk was still observed in 70% of larvae at 11 DPH. Survival varied considerably between replicates and among feeding regimes. Massive mortality was observed at two stages for all feeding regimes/tanks. The first massive mortality was observed right after stocking from hatching tank to nursing tank. About 30-75% larvae died the next day of stocking due to handling. The second massive mortality was observed during 10 to 30 DPH of fry. No mortality was found after 45 DPH and all fish could ingest commercial starter feed (>0.8 mm) for larvae (Purina® AquaMax® Fry Starter 100).

Target Audiences
Current and future yellow perch culturists; culturists of other Percid fishes

Outreach Overview
Since year 1 experiments were repeated this year, year 2 will be completed in 2016 and results will be made available at the end of year 3; both as modules and in a report.

Deliverables (Outputs)
Two videos were produced on “Feeding Yellow Perch Fry” and “Growing & Maintaining Natural Feeds for Larval Fish” by Iowa State University. They are available at https://vimeo.com/127717048 and https://vimeo.com/127639646 . Modules for self/group training will include new procedures/protocols learned from the project.

Outcomes/Impacts
The impacts of this proposed project will be primarily through the development of systems and strategies to enhance mass production and survival of yellow perch from onset of first feeding up to 70 days, the critical period for yellow perch in recirculating aquaculture systems. The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and survival of larval yellow perch by using tank systems and feeding regimes optimized for the early-life stage. At the completion of the project, we expect to not only have information available about recirculating system components, feeding strategies and feed types for successfully reducing the early-life stage bottlenecks, but to have online training modules available through the NCRAC website for aquaculturists in the NCR. The online training modules will provide direct information to yellow perch culturists on current and updated procedures and protocols for reducing yellow perch larval mortality burst in indoor recirculating aquaculture systems.

Impacts Summary

Relevance. — One roadblock hindering expansion of yellow perch aquaculture has been low survival and availability of fry and fingerlings. The survival rate of pond nursed fry is dependent on weather and late winter storms can kill all the fry in ponds overnight. Developing the indoor culture of yellow perch has significant advantages over pond culture. Limiting this possibility has been the poor indoor survival of newly hatched fry to the stage where they are completely feed- trained.

Response. — We have started a project to address the roadblock hindering expansion of yellow perch aquaculture.

Results. — Two commercial-scale algae auto-feeders and rotifer production systems were constructed that allow us to culture and concentrate the needed number of rotifers to feed at a rate and concentration deemed necessary for the amount of fry in each tank. Five feeding regimes or diets were tested for newly hatched yellow perch larvae starting from zero dph up to 80 dph. Non-feeding behavior, non-inflation of the gas bladder, clinging behavior, and cannibalism were examined using a series of methodical experiments. The results have played foundation for completing this project.

Recap. — The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and survival of larval yellow perch by using tank systems and feeding regimes optimized for the early-life stage.

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Yellow Perch activities.

SOME COMMONLY USED ABBREVIATIONS AND ACRONYMS

×	cross, by, or times
AIS	aquatic invasive species
anammox	anaerobic ammonium oxidizing bacteria
AOA	ammonia oxidizing archaea
AOB	ammonia oxidizing bacteria
APHIS	Animal and Plant Health Inspection Service
AREF	Aquaculture Regional Extension Facilitator
AquaNIC	Aquaculture Network Information Center
BOD	Board of Directors
BW	body weight
□C	degrees Celsius
CES	Cooperative Extension Service
COD	chemical oxygen demand
CSFPH	Center for Food Security and Public Health
CVM	Center for Veterinary Medicine
EPC	epithelioma papulosum cyprini
□F	degrees Fahrenheit
FSR	final study report
ft, ft^2, ft^3	foot, square foot, cubic foot
FY	fiscal year
g	gram(s)
gal	gallon(s)
h	hour(s)
ha	hectare(s)
HACCP	Hazard Analysis and Critical Control Point
HCG	human chorionic gonadotropin
IAC	Industry Advisory Council
in	inch(es)
INAD	investigational new animal drug
ISU	Iowa State University
KAA	Kansas Aquaculture Association
kg	kilogram(s)
L	liter(s)
lb	pound(s)
LU	Lincoln University
m, m^2, m^3	meter(s), square meter, cubic meter
MAI	motile *Aeromonas* infection
MAS	motile *Aeromonas* septicemia
MDNRE	Michigan Department of Natural Resources and Environment
µg	microgram(s)
mg	milligram(s)
MC	Mill Creek
min	minute(s)
mL	milliliter(s)
mm	millimeter(s)
MSU	Michigan State University
MT	methyltestosterone
N	number
NAA	National Aquaculture Association
NADA	new animal drug application
NASAC	National Association of State Aquaculture Coordinators
NCC	National Coordinating Council
NCR	North Central Region
NCRAC	North Central Regional Aquaculture Center
NIFA	National Institute of Food and Agriculture
NOB	nitrite oxidizing bacterial
OCARD	Ohio Center for Aquaculture Research and Development
OSU	Ohio State University
oz	ounce(s)
PAH	Phibro Animal Health
PCR	polymerase chain reaction
PFU	plaque-forming units
POW	Plan of Work
ppm, ppt	parts per million, parts per trillion
Purdue	Purdue University
RAC(s)	Regional Aquaculture Center(s)
RAES	Regional Aquaculture Extension Specialist
RAET	Regional Aquaculture Extension Team
RAS	recirculating aquaculture system
RS	Rimler-Stotts
SPAH	Schering-Plough Animal Health
TC	Technical Committee (TC/E = Technical Committee/Extension; TC/R =Technical Committee/Research
™	trademark
TSA	Tryptic Soy Agar
UMESC	Upper Midwest Environmental Sciences Center
USDA	U.S. Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
UW-Madison	University of Wisconsin-Madison
UW-Milwaukee	University of Wisconsin-Milwaukee
VHS	viral hemorrhagic septicemia
VHSv	viral hemorrhagic septicemia virus
WATER	Wisconsin Aquatic Technology and Environmental Research

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